



STITT'S  
DIAGNOSIS, PREVENTION  
AND TREATMENT  
OF  
TROPICAL DISEASES

---

STRONG

## DESCRIPTION OF PLATE OF MALARIA PARASITES

### Benign Tertian Parasites

- 1 Normal red cell for comparison of size
- 2 Trophozoite young ring form
- 3 Trophozoite full grown Red cell is enlarged and Schiffner's dots are present
- 4 Schizont young form undergoing second nuclear division
- 5 Schizont quarter grown Nuclei composed of fine chromatin granules in irregular clumps Yellowish brown pigment is present
- 6 Schizont mature form Nuclear division complete Cytoplasm dividing preparatory to liberation of merozoites
- 7 Macrogametocyte (female gametocyte) Cytoplasm is blue chromatin eccentric compact deep red and surrounded by a halo
- 8 Microgametocyte (male gametocyte) Cytoplasm is greenish blue chromatin central diffuse and light red

### Quartan Parasites

- 1 Trophozoite young ring form Fine black pigment granules are present
- 2 Trophozoite young band or equatorial form
- 3 Trophozoite a more mature oval form showing beginning nuclear division
- 4 Schizont young binucleate form heavily pigmented
- 5 Schizont older band form Pigment is more abundant about periphery
- 6 Schizont mature Chromatin clumps form 8 nuclear masses arranged around central mass of pigment
- 7 Macrogametocyte (female gametocyte) Chromatin is compact and deep red Pigment abundant
- 8 Microgametocyte (male gametocyte) Chromatin is diffuse and pale Pigment abundant.

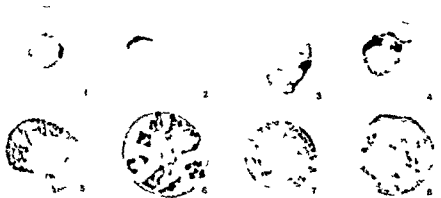
### Malignant Tertian Parasites

- 1 Trophozoite young hair like ring form
- 2 Trophozoites young ring forms Characteristic multiple infection of a red cell showing also peripherally placed forms
- 3 Trophozoite full grown Rarely seen in peripheral blood except in very heavy infections
- 4 } Schizonts in successive stages of maturity Rarely seen in peripheral blood. Specimens from red cells in a brain capillary of a fatal case of cerebral malaria
- 5 }
- 6 }
- Macrogametocyte (female gametocyte) Shows characteristic crescent shape Nucleus is compact deeply stained Pigment clumped in center
- 8 Microgametocyte (male gametocyte) Chromatin is pale staining and diffuse Pigment is dispersed

# BENIGN TERTIAN PARASITES (*Plasmodium vivax*)



## QUARTAN PARASITES (*Plasmodium malariae*)



## MALIGNANT TERTIAN PARASITES (*Plasmodium falciparum*)



(L. S. vol. II, p. 100)

(A. D. W. vol. I)

## MALARIA PARASITIS

U. S. G. P. O.



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# STITT'S DIAGNOSIS, PREVENTION AND TREATMENT OF TROPICAL DISEASES

By RICHARD P. STRONG, M.D., Sc.D., D.S.M., C.B.  
Professor of Tropical Medicine Emeritus, Harvard  
University; Consultant in Tropical Medicine to the  
Massachusetts General Hospital and the Boston City  
Hospital; Member of the Health Council, Commonwealth  
of Massachusetts; Trustee of the Carnegie Institution,  
Washington; Colonel, M.C., United States  
Army; Consultant to the Secretary of War and Director  
of Tropical Medicine, Army Medical School,  
Washington, D.C.

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SEVENTH EDITION  
*In Two Volumes*  
VOLUME ONE

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THE BLAKISTON COMPANY  
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*First Edition* November 1914

*Second Edition* August 1917

*Third Edition* February 1919

*Fourth Edition* August 1922

*Fifth Edition* February 1929

*Sixth Edition* January 1942

*Reprinted* January 1943

*Reprinted* March 1943

*Reprinted* December 1943

*Seventh Edition* October 1944

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PRINTED IN U S A

BY THE MAPLE PRESS COMPANY YORK PA

## PREFACE TO THE 7TH EDITION

The necessity for a new edition emphasizes anew that under the greatly changed conditions of this world and the increased opportunities for the aerial transportation of disease the medical profession has come to recognize the growing importance of tropical medicine and its worldwide significance and feels that further knowledge and especially the dissemination of new information regarding it is desirable

The Honorable Secretary of War Henry L. Stimson in a message sent to a class of medical officers graduating recently at the Army Medical School Washington D C emphasized that there is no field of medicine more important to the United States Army in this war than tropical medicine and our troops have been headed toward a multitude of places where they should not go without doctors trained in combating diseases peculiar to the tropics

Major General Norman T. Kirk Surgeon General of the United States Army in his commencement address at the graduating exercises of the 16th class in Tropical and Military Medicine at the Army Medical School Washington D C on December 18th 1943 pointed out that there is little tropical medicine and sanitation taught in our medical schools today nor has it ever been taught in the majority of these schools to the extent that would train and prepare satisfactorily military medical officers for their work at the present time when our Army is scattered throughout the world especially where tropical diseases are such a problem Our biggest problem in the Army today from a medical standpoint is malaria and other tropical diseases The non effective rate due to disease is six or seven times as great as battle injury

Vice Admiral Ross T. McIntire Surgeon General of the United States Navy in a recent address summarized his views with the statement the entire field of tropical medicine is one that is so important that we should make every attempt to interest all groups of professional men in this specialty and he added the time is not far distant when any doctor who does not have a clear picture of the dangers of tropical diseases and an understanding of their diagnosis and treatment will drop behind his fellows. Rear Admiral E. R. Stitt United States Navy Surgeon General (Ret) in his recent inspiring lectures both at the National Naval Medical Center and at the Army Medical School has also emphasized the greatly increased importance of the subject.

Therefore the writer has again endeavored to include additional researches or publications made since the last reprint At the Army Medical School he is especially indebted to Colonel George R. Callender M C United States Army Director of the Army Medical School and Assistant Commandant of the Medical Department Professional Service Schools Washington D C and to Lieutenant Colonel Thomas T. Mackie M C Army United States Executive Officer Course in Tropical

and Military Medicine and Chief of the Division of Parasitology Army Medical School for suggestions and for the inspiration he has received from them in the publication of this revision.

Also he wishes to thank Major George W. Hunter, III, Sn C., Army United States, Captain C. Brooke Worth M C. Army United States, and Captain Gordon I. Davis Sn C. Army United States and the other members of the Division of Parasitology of this School for assistance in the editorial work.

Commander James J. Sybero, M C. United States Navy, Malina and Epidemic Control Officer in the South Pacific, has given the writer much recent information concerning the subjects of dengue fever, scrub typhus and filariasis in the South Pacific and Captain Eric G. Hakansson, Medical Officer in Command, Naval Medical Research Institute, National Naval Medical Center, has further helped with reference to intestinal infections.

The writer also wishes to thank Doctor Henry I. Meleney, Professor of Preventive Medicine at New York University and Chairman of the Sub Committee on Tropical Diseases, National Research Council, and Doctor Wilbur Sawyer, Director of the International Health Division of the Rockefeller Foundation and a member of the Sub Committee on Tropical Diseases, National Research Council for further recent information regarding the subject.

He in addition wishes to express his appreciation to his British colleagues Major General Alexander Gordon Hoggan M D, F R C P, D I M & H R A M C K I P Brigadier N. Hamilton Laibley C B I M D D S c I K C I and D I M A A M C and to Colonel I. S. Gillespie B A M D K A M C British Liaison Officer Medical Field Service School, Carlisle Barracks for much information concerning tropical disease in the British and Australian Armies. Also he is grateful to Sir Philip Manson-Bahr C M C D S O K A M C M R C S, D I M & H I / S for his suggestions and interest in the publication. The Tropical Diseases Bulletin London under the Chairmanship of Doctor A. C. H. Smart C M G M B E M D D P H D I M & H is of continued valuable assistance and has maintained its exceedingly high standard in spite of war conditions.

The writer is much gratified to have received reports of the manner in which the book has been made use of by many students and physicians in Civil life as well as by medical officers of the Army Navy and Public Health Services both in the United States and abroad.

Finally he feels that he should, and wishes to add that his greatest inspiration and incentive for this work has come from the desire of his late beloved wife Grace Nichols Strong, that this book should prove to be useful both for instruction in our medical schools and in the advancement of our knowledge of tropical diseases and in the relief of suffering from these afflictions.

RICHARD I. STRONG

## PREFACE TO THE 6TH EDITION

Since the publication of the last edition of this text book in 1929 by E R Stitt M D Sc D LL D Rear Admiral Medical Corps and Surgeon General U S Navy Retired knowledge regarding many of the diseases encountered more commonly in tropical countries has been very greatly increased So that in order to review this subject and incorporate the important scientific progress that has been made it has been thought advisable to rewrite many sections of the text In the present edition it has been the aim to make available a summary of the knowledge not only of the clinical manifestations regarding tropical diseases and their treatment but also of whatever zoological aspects and laboratory measures as are of importance in connection with their transmission diagnosis and prevention Also brief consideration has been given to the more important cosmopolitan diseases that may be encountered in warm countries

There has been so much discussion recently regarding the term Tropical Diseases that the writer feels it desirable to explain that the diseases specially discussed in this text book under this term occur commonly or most frequently in tropical countries although many of them are encountered from time to time and some are even endemic in countries with temperate climates

Progress in the medical sciences has been so great during the past decade that the student and medical practitioner today frequently desire a more comprehensive discussion of the subject of disease in its different aspects than in earlier years In order to incorporate the facts which are of interest and useful both from a clinical and laboratory point of view it has been necessary to increase the size of the book Although this is in some respects deplorable nevertheless there is the advantage that the reader will find available in the two volumes a discussion of all these different aspects of the subject

The writer has endeavored to avoid dogmatic discussions and has tried to present the available evidence and express differences of opinion where such exist that the student may not be led to believe there is but one point of view For the reader who wishes more extended information a brief list of references has been appended to each chapter These lists of course are not intended to cover the subject but merely to refer students to some of the more recent investigations or specially important earlier ones should they desire to read the articles in the original and more extended form

Since the passage in 1935 in the United States of the Social Security Act with its health provisions the increased emphasis that has been placed on preventive medicine public health and sanitary engineering has demonstrated the importance of information regarding these subjects to the general physician and for this reason in the present edition considerable attention has been devoted to the public health problems regarding the prevention of the infectious diseases discussed



The last year has demonstrated the steadily increasing interest in Latin America not only in its political and economic aspects but in its cultural and social values as well. Also the policy of the United States to increase friendly relations with Latin America and the activities of the Carnegie Endowment for National Peace and the Pan American Sanitary Bureau and the appointment of Medical and other Fellows by the Guggenheim Foundation from several Central and South American republics have emphasized the importance of Pan American unity and of many of the health problems of mutual interest. Obviously the greater portion of South America is within the tropics and the increased amount of travel between these countries especially by air makes the problem of the treatment of tropical diseases particularly important to physicians in the United States as well as to those in Central and South America. In the pre ent edition attention has been given both to the occurrence and prevalence of the diseases in Central and South America and to many of the recent important investigations which have been carried out there upon tropical medicine.

The writer wishes to avail himself of this opportunity to express his appreciation and thanks to Surgeon General Hugh S. Cumming, First Vice President of the League of Nations Health Association and Director of the Pan American Sanitary Bureau Washington D C and to Dr Yves M. Biraud head of the Services of Epidemiological Intelligence and Public Health Statistics of the League of Nations for their courtesy in granting permission for the reproduction of a number of the maps showing the geographical distribution of disease and recently prepared by the League of Nations.

In particular the writer is indebted to Lieut James J. Sapero Medical Corps U S Navy for the preparation of the frontispiece Plate I (The Malaria Parasites) and Plate III of the malaria parasites in thick blood films and in cerebral malaria drawn from original preparations in the collection at the Naval Medical School. Likewise I am very appreciative of the courtesy of Commander E. G. Hakansson Medical Corps U S Navy for Plate IV (Intestinal Amoebae and Flagellates drawn to scale) of preparations carefully selected by him from the splendid collection in the school museum and for a number of valuable suggestions in connection with zoological studies regarding amoebae.

Dr George C. Shattuck Clinical Professor of Tropical Medicine Harvard University has written the chapters upon Nutritional Disorders and upon Heat Stroke and has revised and made additions to the important section on Tropical Hygiene. Dr A. W. Sellards Richard Pearson Strong Associate Professor of Tropical Medicine Harvard University who has been concerned with investigations upon yellow fever since 1927, has kindly written the chapter on this subject. Dr J. H. Sandground formerly helminthologist in the Department of Tropical Medicine at Harvard University and now of the Biological Division at the Lilly Research Laboratories has read the proof of a number of the sections on parasitology and Dr Joseph C. Bequaert Assistant Professor of Comparative Pathology and Tropical Medicine Harvard University has read

the manuscript of the book and given special suggestions with reference to the entomological sections. Dr Thomas R. Barbour, Director of the Museums of Harvard University, including Comparative Zoology, has read the chapters on poisonous snakes and lizards and poisonous arthropods, fish and coelenterates and has written in addition some observations upon poisonous fish. I am also much indebted to Dr Reginald Fitz, Lecturer on the History of Medicine at Harvard, for reading the chapter on Black water fever and for suggestions in connection with it. In the revision of the chapter on Dengue fever the writer has been especially guided by the investigations and publications of Col. James S. Simmons, United States Army Medical Corps, while Col. George R. Callender of the Medical Corps, Director of the Army medical school, has given valuable information in regard to instruction and investigations particularly at this school. Through the good offices of these gentlemen the value of the book obviously has been much enhanced.

The writer is also grateful for the kindness extended by the Executive Committee of the Royal Society of Tropical Medicine and Hygiene in permitting the reproduction from the Transactions of several tables and illustrations and by a number of physicians and publishers of other medical journals who have permitted the reproduction of other illustrations which are all acknowledged in the text. In addition he particularly wishes to express his thanks for the courtesy of Messrs. D. Appleton, Century Company, Inc., publishers of *Billings-Forscheimer Therapeutics of Internal Diseases* and *Bedside Diagnosis*, George Blumer Editions, and of Messrs. Thomas Nelson & Sons, publishers of *Nelson Loose Leaf Medicine* in regard to earlier articles written by the undersigned and published by them.

The writer has also been much aided in the preparation of the volume by the work of Col. Charles F. Craig and Dr Ernest C. Faust, published particularly in *Clinical Parasitology*, 1940, Lea & Febiger, and of Dr Asa C. Chandler in *An Introduction to Parasitology with Special Reference to the Parasites of Man*, 1940, John Wiley & Sons, Inc., and of Dr Philip Manson-Bahr in *Manson's Tropical Disease*, 1940, Williams & Wilkins Co. The *Tropical Disease Bulletin*, London, with its valued reviews has also been of much assistance. Finally, in the present edition wide use has been made of the valuable work of Stitt, Clough and Clough, *Practical Bacteriology, Haematology and Parasitology*, The Blakiston Company, 1938. Dr Clough has kindly read the section on anaemia and most valuable counsel throughout has been given by Admiral Stitt.

Especial thanks are due to The Blakiston Company for their interest, intelligent cooperation and assistance in the publication of the work. I am also appreciative of the great assistance of Mr. J. Tuckermann Day of the Riverside Press, Cambridge, in the preparation of the index.

President Franklin D. Roosevelt, in an address made at the National Institute of Health on November 1, 1940, emphasized that the United States was less than a day by plane from the jungle type of yellow fever of South America, less than two days from the sleeping sickness of Equatorial Africa.

The last year has demonstrated the steadily increasing interest in Latin America not only in its political and economic aspects but in its cultural and social values as well. Also the policy of the United States to increase friendly relations with Latin America and the activities of the Carnegie Endowment for National Peace and the Pan American Sanitary Bureau and the appointment of Medical and other Fellows by the Guggenheim Foundation from several Central and South American republics have emphasized the importance of Pan American unity and of many of the health problems of mutual interest. Obviously the greater portion of South America is within the tropics and the increased amount of travel between these countries especially by air makes the problem of the treatment of tropical diseases particularly important to physicians in the United States as well as to those in Central and South America. In the present edition attention has been given both to the occurrence and prevalence of the diseases in Central and South America and to many of the recent important investigations which have been carried out there upon tropical medicine.

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## FOREWORD

For many years I have been asking Doctor Strong why he did not write a book on tropical medicine and he has always replied because you have written a book which seems to have covered the ground to my satisfaction

Doctor Strong and I have been close friends for almost forty years dating from the early years of American occupation of the Philippine Islands

From 1909 to 1911 I was a member of the staff of The Department of Tropical Medicine of the Medical School of the University of the Philippines of which he was the head In addition he was Chief of the Biological Division of the Philippine Bureau of Science in which capacity he was responsible for important research in the problems of tropical medicine Prior to his resignation from The Medical Department of the U S Army he served as President of the Army Tropical Disease Board (1899 to 1901) In later years such distinguished officers as Nichols Siler Craig Ashburn Dunham and other Army officers have served on this board

I doubt whether any worker in the national or international field of medicine has equalled Strong as director of commissions to study problems connected with public health in the tropics and elsewhere As Chairman of Red Cross commissions he investigated pneumonic plague in Manchuria typhus fever in the Balkans and trench fever in the allied troops in France In addition he has headed commissions to study health conditions in Liberia for Firestone and other Harvard University research expeditions in South and Central America More recently he has been particularly identified with studies of bartonellosis in Peru and onchocerciasis in Guatemala

In the first five editions of this book I had the fullest aid from medical officers of the Navy and the Public Health Service The fifth edition of which this is a revision was brought up to date by the painstaking review of the literature by Doctor John Harper and Doctor Paul Dickens both of the Medical Corps of the Navy Dickens changed the clinical section of the fourth edition from a regional presentation to an alphabetical one Doctor Strong has decided to retain the alphabetical order as more available for quick reference

Doctor Montgomery Stuart, who had been health officer of Haiti prepared the section on Tropical Hygiene which has been revised by Strong Shattuck and his colleagues To Admiral Butler and Captain Bunker of the Navy and to Doctors McCoy and Francis of the Public Health Service I was indebted for notes and advice With every one of

tional Africa less than three days from cholera and bubonic plague, and he added, The ramparts we watch must be civilian in addition to military. Hence the world now, has become so inter related and apparently so small that our medical and sanitary responsibilities regarding tropical diseases have greatly increased.

Dr Lewis L. Williams, Jr. President of the American Society of Tropical Medicine in 1940 has called attention to the importance that tropical medicine assumes in the event of a national emergency and pointed out that with the drafting of a large army of young men their encampment in the southern United States together with the establishment of American bases in the Caribbean and the disturbed conditions in the Orient diseases associated with warm climates assume added importance. The present war has likewise emphasized anew the importance of tropical diseases in the Near East and Africa.

The remarkable excellence and success which previous editions of this text book written by Admiral Stitt, have attained are well recognized by practically all workers in the field of tropical medicine. The writer realizes the difficulties, or perhaps the impossibility of attaining the success reached in previous editions. Nevertheless he has felt the task to be imperative that this American text book on the subject should be retained and that the present knowledge of the subject should be summarized and made available at this time of emergency.

HARVARD UNIVERSITY

August 1941

RICHARD P. STRONG

The demand for the 1942 edition of this work has exhausted the issue and a reprint has become necessary. The writer feels deeply indebted to the members of the medical profession who have expressed their approval of the book and for the support and interest they have displayed in it. In connection with this reprint he wishes to add his thanks especially to Colonel James S. Simmons, Chief Preventive Medicine Division, Office of The Surgeon General, United States Army and to Colonel George R. Callender, Director of the Army Medical School and Assistant Commandant, Army Medical Center for much advice and assistance. He is also very grateful to Lt. Colonel W. Komp, Senior Medical Entomologist, United States Public Health Service and to Lt. Colonel Paul F. Russell, Division of Preventive Medicine, Office of The Surgeon General for much advice and help in the classification and identification of mosquitoes particularly in regard to the prevention of malaria.

In the discussion of tetanus he is also much indebted to Colonel Stanhope Bayne Jones, Assistant Director Preventive Medicine Division, Office of The Surgeon General, whose recent investigations and experimental work have led to a modification of some of the previous ideas regarding this disease. The writer is also very grateful to Admiral F. R. Stitt and Captain E. G. Hakansson, Bureau of Medicine and Surgery, United States Navy for their continued interest in the book.

In this reprint the important investigations carried out especially during the past year have been added.

ARMY MEDICAL SCHOOL

December 5 1942

RICHARD P. STRONG

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the first five editions I had the assistance of Rear Admiral H W Smith of the Naval Medical Corps

This new (sixth) edition is really a new book largely rewritten and made a book of reference by quoting recent research, and presenting a rather complete bibliography Furthermore the prevention of disease has been stressed so that the title of the book has been changed from

Diagnosics and Treatment of Tropical Disease to Diagnosis Prevention and Treatment of Tropical Diseases

The new edition is the work of Doctor Strong and his colleagues in the Department of Tropical Medicine of Harvard University

In 1931 it was necessary for me to prepare a new edition of the companion volume to this manual— Practical Bacteriology, Haematology and Animal Parasitology I soon found that I lacked the energetic enthusiasm for new things so necessary for keeping abreast of research work and best retained when one is in touch with students I was fortunate in obtaining the cooperation of Doctor Paul W Clough and Doctor Mildred C Clough of Johns Hopkins University and, with the rewriting of this book, I have been equally fortunate in persuading Doctor Strong to take on the revision

E R STITT

WASHINGTON D C

August 1941

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## SECTION I

### DISEASES DUE TO PROTOZOA

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#### Chapter I

#### MALARIA

From the standpoint of prevalence malaria appears to be the most important of all diseases in the world today. In a study by the Health Organization of the League of Nations into *The Quinine Requirements of Malarial Countries and the World Prevalence of Malaria* (1932) the reports from 65 countries showed that a total of 17 750 760 cases of malaria were treated during the year of the study. The proportion of cases of malaria treated to the population and to the total number of individuals with other diseases treated affords a valuable index of the prevalence of malaria in countries in which a highly developed medical service exists. However the number treated obviously does not give a complete idea of the number actually existing and in certain tropical countries it is impracticable to make complete surveys. Nevertheless we know that in Indo-China Ceylon the Straits Settlements and the Federated Malay States malaria alone has accounted for from at least 10 to 56.9 per cent of the cases of infectious disease treated in hospitals or dispensaries.

The number of cases treated also reflects the extent of the anti malarial work conducted in some countries especially when the quantity of quinine dispensed is taken into account. In India for instance out of 100 000 000 malaria cases which is considered by the Malarial Commission to be a moderate estimate only from eight to ten millions were treated the quantity of quinine distributed not exceeding an average of 2 grams per annum per case. The condition in India is cited because it is the largest of the malarial countries (with a population of some 353 000 000) for which fairly full particulars have been obtainable but there are a number of other countries in which the prevalence of the disease and the conditions of treatment are somewhat similar. Other figures show that more than three million deaths from fever have been notified every year in India. The severity of malaria is also emphasized by the virulent outbreak which occurred in Ceylon in 1934-35 in which 66 704 persons were reported to have succumbed. Its prevalence in British colonial territories is shown by the report of Granville Edge (1937) that during 1935 among some 61 000 000 inhabitants more than 6 500 000 cases of malaria were treated at the various medical centers controlled by the several colonial govern



from the bite of such an infected mosquito. Clinically malaria is characterized by periodic attacks of fever associated with anaemia and enlargement of the spleen and if untreated with cachexia and a deposit of black pigment in the various organs. The malady is amenable to treatment with quinine and several other synthetic compounds inimical to the life of the parasite.

At least 5 species of the genus *Plasmodium* are recognized as pathogenic for man, the 3 important species being (1) *Plasmodium vivax* giving rise to tertian or benign tertian malaria, (2) *P. malariae* to quartan malaria, (3) *P. falciparum* to malignant tertian (subtertian or aestivo autumnal) fever. A fourth and much rarer species *P. ovale* having several features in common with *P. vivax* and *P. malariae* produces a very mild form of tertian malaria in man. In addition a fifth species *P. knowlesi*, normally a parasite of the rhesus monkey, has on many occasions been successfully inoculated into man, particularly in the treatment of general paresis, with the production of malarial attacks usually of a mild character.

The validity and pathogenesis of these species has been proved not only by their distinctive morphologic characters but also by direct inoculation into man and by their mosquito transmission.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History**—Malaria was formerly supposed to be due to poisonous emanations from damp ground, hence the term malaria introduced into English literature about 1829. Hippocrates 460-370 B.C. in his book on epidemics noted the existence of periodic fevers, divided them into quotidian, tertian, quartan and subtertian and referred to the enlarged spleen. Celsus recognized 2 types of tertian fever, one benign and similar to quartan fever, the other in which the attack is of longer duration and far more severe in character, the fever occupying 36 of the 48 hours and not entirely subsiding in the remissions but being only mitigated.

Columella about 16 B.C. suggested that the virus of malaria emanated from marshes and associated the disease with insects originating in them which attacked man in swarms. Also in the time of Caesar, views were expressed by Varro that swamp air might be the cause of malaria and furthermore that animals so small that the eye could not follow them might transmit diseases by way of the mouth or nose. In view of our present knowledge it is remarkable that Lancisi in 1718 should have associated marshes with the development of gnats, which insects he thought could not only introduce with their proboscides the putrifying organic matter of such swamps but animalcules as well.

In 1638 Countess del Chinchon, wife of the Viceroy of Peru, was cured of an intermittent fever by treatment with the bark of a certain tree, which bark was introduced into Europe in 1640. Linné, who named the genus of quinine-producing trees about 100 years later, left out the first *h* in the name, hence the mistaken spelling *Cinchona*.

While Morton and Sydenham in 1666 noted the specific action of cinchona in differentiating certain fevers, it remained for Torti in 1712 by the use of this drug to differentiate more completely those fevers which were cured by cinchona from those which failed to yield to this specific. In giving the drug Torti used large doses, the first 3 days. After that he administered smaller doses for 2 or 3 weeks. Quinine was not

ments and that this disease alone was responsible for approximately 25 per cent of the total cases among in patients and out patients treated for all causes of ill health

In the western hemisphere in many areas in South America where malaria prevails so widely it is still the disease which occasions the greatest mortality. The Oswaldo Cruz Commission reported that in the regions along the Rio Negro Brazil it was difficult to find a single individual who did not show signs of chronic malarial infection. Souza Araujo regards the depopulation of many regions in Amazonas as largely due to the great mortality from malaria.

In the southern United States, also where we have no very reliable records of the number of malaria cases that exist, the malaria mortality rate has been reported by Faust (1938) as higher than it was ten years ago and he believes that malaria is extending in this country from heavily endemic foci into areas not significantly malarious a decade ago.

The Malaria Committee of the Pan African Conference, at the end of 1935 emphasized that, with the exception of certain highland areas and a large region comprising the more southerly parts of the Union of South Africa malaria occurs throughout almost the whole of the African continent and affects many millions of the population. It occupies one of the foremost places—if, indeed not the foremost—among the infective diseases of Africa as a cause of mortality and morbidity in the indigenous population, and as such it plays an outstanding part in hindering the progress and social development of these peoples and in retarding the advancement of industry and trade.

Boyd (1939) points out that to the welfare of the human race malaria presents a problem of the first magnitude whether considered from the viewpoint of range of distribution of morbidity or of mortality. As a cause of morbidity it is the peer of all other infections and as a cause of mortality it is formidable. Hence malaria, which has for centuries held first place in prevalence and importance among the general communal diseases must still be considered as one of the most important from both a medical and a public health standpoint. Hackett and Russell (1938) stress the fact that rural malaria especially in the tropics is one of the principal unsolved problems in the field of public health.

#### SYNONYMS AND DEFINITION

Synonyms—Marsh miasma Remittent fever Intermittent fever  
Ague Paludism Jungle fever French *Paludisme* German  
*Wechselfieber*

Definition—Malaria (from the colloquial Italian *mala'* bad and *aria* air) is an infection characterized by certain febrile disturbances caused by protozoan parasites of the class SPOROZOA and of the family PLASMODIIDAE. Man is the intermediate host of these parasites which undergo an asexual stage of development in the red corpuscles. The parasite undergoes a sexual phase of development in the *Anopheles* mosquito which is hence the definitive host. Man acquires infection

from the bite of such an infected mosquito. Clinically malaria is characterized by periodic attacks of fever associated with anaemia and enlargement of the spleen, and if untreated with cachexia and a deposit of black pigment in the various organs. The malady is amenable to treatment with quinine and several other synthetic compounds inimical to the life of the parasite.

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extracted and introduced into practice until after 1820. Audouard in 1803 was the first to draw definite attention to the splenic enlargement of malaria.

Nott 1848 and Beauperthuis 1853 expressed views as to the transmission of malaria and yellow fever by insects and Robert Koch also appears to have conjectured that the infection could be caused by the bite of a blood sucking insect. Indeed the natives of parts of Africa applied the same name (*Mbu*) to the insect and to the disease.

In 1847 Meckel announced that the dark color of malarial organs was due to a pigment and in 1848 Virchow noted that this pigment was intracellular. In 1875 Kelsch observed pigmented bodies in malarial blood and in 1880 came to the conclusion that these pigmented cells were diagnostic of malaria.

*The Discovery of the Parasite*—The year 1880 was a most important one in the history of malaria for on November 6 1880 Laveran at Constantine first recognized the parasites of malaria while carrying on investigations as to the origin of the 'pigmented bodies' and pigmented leucocytes. He observed not only spherical pigmented bodies but also crescents and in particular the flagellation of the male gamete, which demonstrated to him that these were living organisms. He proposed the name *Oscillaria malariae* on account of the movements of the flagellate body but this had to be dropped as not valid the generic name *Oscularia* having been previously applied to another organism.

When these bodies were demonstrated to various Italian authorities in 1882 they were thought by them to be degenerated red cells. At this time the Italians influenced by the work of Pasteur were convinced that an organism *Bacillus malariae* reported by Klebs and Crudeh (1879) to have been isolated from water and soil of malarious districts was the cause of malaria. This bacillus was said to be cultivable on ordinary media and to be capable when injected into man of producing malaria.

By 1885 the Italians became convinced that the bodies discovered by Laveran were the cause of malaria and Marchiafava by staining with methylene blue noted the ring forms and their increase in size up to that of the sporulating parasites. Colgi discovered not only that the malarial paroxysm coincides with the period when the sporulating forms (merocytes) simultaneously reach maturity but also the exact working out of the cycle of quartan malaria. He even showed three stages of development of the parasites in a triple quartan. To Golgi Marchiafava and Celli we owe our first knowledge of the existence of different species of parasites for different kinds of malaria. In these investigations they showed that as a rule a certain type of malaria could be produced by injecting the blood of such a case of malaria into a well man. Gerhardt in 1884 was the first to produce malaria by the injection of malarial blood. At this period a great deal of research was carried on as to the origin of malarial parasites and it was found that many animals harbored parasites somewhat similar to the malarial parasites of man. In 1891 the chromatin staining method of Romanowsky was introduced which by bringing out the variations in chromatin distribution led to more accurate study of species and cycles.

Our present exact knowledge as to the existence of 3 common species of malaria is largely due to the careful examinations made by Koch of fresh and stained malarial blood preparations.

*Mosquito Transmission*—In 1894 Manson formulated the hypothesis of the mosquito transmission of malaria. He based this upon facts he observed in tracing the life history of filaria and upon the fact that in malaria the flagellation of the male gametocyte does not take place for several minutes after the removal of the blood from the peripheral circu-

lation. He also suggested that larvae might feed upon infected mosquitoes dying upon the water and thus acquire the disease.

Ross for 2 years caused mosquitoes to feed upon the blood of malarial patients which contained crescents but as he used insects of the genera *Culex* and *Aedes* no development of the parasites in the tissues of the mosquitoes occurred. In 1897 he used eight dappled wing mosquitoes (*Anopheles stephensi*) and in 2 of these upon dissection he noted the development of the pigmentary bodies to be different from anything he had observed in hundreds of dissections of other mosquitoes.

In 1886 Metschnikoff from observation of sporulating parasites in the brain capillaries at the autopsy of a malarial case considered them to be coccidial in nature. In 1892 Pfeiffer studying the *Coccidia* showed that there was an endogenous cycle going on in the epithelial cells as well as the long known exogenous cycle connected with the ingestion of oocysts passing out in the faeces of an animal infected with coccidiosis. He suggested that malaria might similarly have an exogenous cycle as well as the well known endogenous one. Opie noted hyaline and granular forms of parasites in the blood of crows and MacCallum working with this malaria like disease of birds (*Halteridium*) observed the fecundation of a granular female parasite by the flagellum like process of the hyaline male cell.

In 1898 in India working with a malarial disease of sparrows (due to *Plasmodium praecox*) Ross infected 22 out of 28 healthy sparrows by mosquitoes of the genus *Culex* which had previously fed on sick sparrows. He noted in the culicine mosquito employed for transmission the complete cycle of development of the parasite and this cycle was subsequently worked out for human malaria in anopheline mosquitoes (*Anopheles maculipennis*) by Grassi and Bignami in Italy.

Koch demonstrated that the malaria like infections of other animals had no part in the causation of human malaria and that the malarial parasite could only circulate between man and certain mosquitoes.

In order to demonstrate conclusively the connection between infected mosquitoes and malaria Sambon and Low lived for 3 of the most malarious months of 1900 in one of the most malarious sections of the Roman campagna in a mosquito screened hut and did not contract malaria. Infected mosquitoes were also sent to London from Italy and allowed to feed upon Dr P. T. Manson and Mr George Warren. After a period of incubation these volunteers came down with typical malaria with parasites in the blood. As Manson has stated from these scientific observations the mosquito malaria theory passed from the region of conjecture into that of fact.

**Geographical Distribution and Prevalence**—Malaria is widely distributed over all parts of the tropical and subtropical world and over many more temperate regions as is illustrated roughly in Fig. 1. It is prevalent between 45° N and 40° S latitude. However the indigenous malaria belt in the different continents may be said to extend from 60° N in Europe (Lake Ladoga, Russia and southern Sweden) to 30° S in Africa.



(Natal), to 40 S in South America (Argentina), and rarely to 20°S in Australia (Queensland). The prevalence of the different species varies somewhat geographically. *Plasmodium vivax* is the most widely distributed through these areas and is the prevailing species in the temperate zones. *P. malariae* is comparatively rare. It is more a parasite of temperate and subtropical areas than of the true tropics, but in both it has a very limited distribution. *P. falciparum*, the subtertian or malignant tertian parasite, is especially encountered in badly infected districts in the warmer parts of the world hence the name tropical malaria. It is the prevailing species in India, southern China and Central Africa. It is much rarer in temperate climates as in northern Europe.

In Europe, although thousands of malaria carriers returned to England from war areas, and *A. maculipennis* (the common transmitter in Europe) is prevalent in many sections of England, there have been very few indigenous malarial infections reported, except in the low lying parts of Essex and Kent. The mean temperature of England being too low for the development of malignant tertian zygotes, benign tertian (*P. vivax* infection) is the only form of malaria to be suspected as originating in that country. Patients with malignant tertian malaria seen in England must have contracted the primary infection elsewhere.

In North Holland, benign tertian malaria is an important disease. In Holland the transmitting mosquito *A. maculipennis* is reported to prefer animal blood, so that during the warmer season it frequents cattle barns while in the colder months it returns to human habitations where it remains constantly, and conveys infections actively until December.

Hence destruction of female *Anopheles* found in houses during the winter is recommended as the best measure of protection. Swellengrebel and deBuck (1938) emphasize that the malaria problem in the Netherlands is now serious as each new territory reclaimed from the sea adds a new focus and the mosquitoes breed profusely in the ditches draining the land which is low and full of canals. A short winged race of *maculipennis* (*atroparvus*) the vector of most importance breeds best in water of rather high salinity. It is believed satisfactory control will probably come only when the canals are so diluted and flushed with fresh water that this short winged anopheles is largely eliminated.

Severe epidemics of malaria have occurred in Denmark and in northern Germany in earlier years. Other important centers for malaria in Europe are southern Spain and Italy and the islands of Corsica and Sicily.\*

During the World War I the Balkan States and especially Macedonia were dangerous centers for malaria. The mosquito hosts were *A. maculipennis* and *A. superpictus*. All 3 species of malaria were reported but it was the malignant tertian infections which gave rise to so much serious illness in Macedonia.

Salonica and the Danube marshes were also highly malarious. Russia is intensely malarious especially in the southern parts. Taraskevitch reported infections above 15 per cent even in Petrograd where *A. maculipennis* seemed to be the carrier. Drboblav (1936) has also reported its prevalence in Hungary and in sub Carpathia from 1925 to 1936.

Pampuna states that Greece especially Ipirus is the most malarious country in Europe.

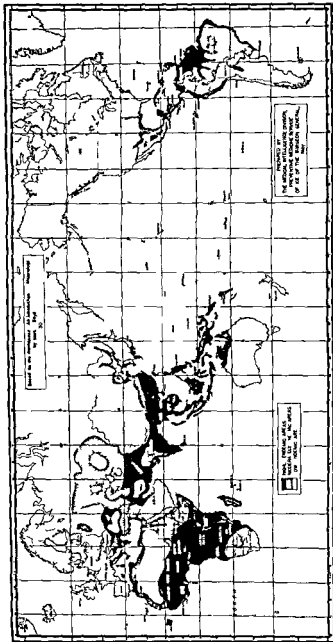


FIG 1—Graph of malaria distribution in the Pacific (Courtesy of Preventive Medicine Branch, U.S. Army Medical Service, 1942)



In Asia Minor especially Palestine malaria has prevailed extensively for many years and during the War the control of this infection became a great problem. There were many anopheline species and all types of malaria were present. Benign tertian was prevalent from February to June and malignant tertian from October to December. Reports gave about 5 per cent of infection for the Jews and 15 per cent for the Arabs. In recent years *Anopheles elutus* has been the commonest transmitter in Palestine (Barber and Rice 1935).

While we have no accurate statistics regarding malaria in China Gear (1940) has found from a survey that every hospital in China reported the presence of malaria.

Williams (1941) reports that malignant tertian malaria has been very prevalent in Yunnan Province since 1935 and in the summer of 1939 made disastrous inroads on hundreds of thousands of Chinese employed as laborers on the China Burma highway.

In the United States the principal endemic area is in the southeastern states east of the line marking the mean annual precipitation of 30 inches (Boyd 1939). Toward the interior it is largely delimited by the division between the Atlantic coast plain and the Piedmont plateau but it has extended in the past along the Atlantic coast as far north as Boston. In the northern portion of this range except in imported cases tertian malaria has been almost the sole form encountered, a few cases of quartan occurring. South of Chesapeake Bay much aestivo-autumnal malaria has been observed. Malaria does not appear to be normally endemic in the Piedmont plateau although extensive epidemics have been observed about the hydro-electric impoundings, a cause recently of considerable concern to public health authorities.

In earlier years the endemic area extended considerably to the north occupying the whole of the upper valley of the Mississippi and southern Canada as far north as the summer isotherm of 70 F. During the past fifty years however the disease has almost entirely receded from this area. Minor endemic areas are still to be found in the irrigated regions of New Mexico, the central valley of California, the valley of the Sacramento as far as 40 N. and the valley of the Columbia River. In California in 1938 358 cases were reported and in 1939 284 cases (Reed 1940).

In the endemic areas in the southeastern states fairly intense epidemics of malaria still occur at irregular intervals during the summer and autumn months. They vary greatly in severity yet may be very prostrating to a community and may cause considerable mortality usually due to the aestivo-autumnal parasite.

Extensive anti malaria operations in the last 15-18 years have eliminated endemic malaria from most cities and towns of this area but it continues to be a very important medical and public health problem in many rural communities and small towns as emphasized by Meleney (1937) and Boyd (1939).

It is unfortunate that we have no accurate statistics of the cases of malaria which occur annually in the United States. Only with reference

to the mortality of malaria in the southern states can we obtain some idea of its prevalence and seriousness. Chandler (1940) estimates from the death rate that there may perhaps have been a million cases in the United States in 1936, on the basis that there are from 100 to 500 cases of malaria for each death, and the Metropolitan Life Insurance Company estimated that there were 900 000 cases in the United States in 1935. In the United States in 1934 Meleney reported that there were 3900 deaths from malaria in 13 southern states, and in 1935 there were 4435 deaths. In 1935 according to McKinley in 15 states there were 54 300 cases of malaria reported which however, gives little idea of the number of cases of the disease that existed. In 11 of the southern states malaria still remains a major public health problem and in a few a leading cause of death.

Faust (1940) has summarized the reports of the Departments of Health of the malaria mortality of fourteen of the southern states with a population approximating 35 000 000. This mortality has varied considerably in the different states in different years. Thus in 1935 the average mortality was 11 per 100 000, or 4.4 per cent higher per 100 000 than in 1931. However in 1938 the death rate had fallen again to 5.8. In some of the states there was an increased mortality, in others a decreased one.

Thus in 3 states—Georgia, North Carolina and Oklahoma—there was an increased prevalence in 1936. The disease was apparently more prevalent in the coastal portions of Georgia, South Carolina, North Carolina and Virginia, in the southern half of Alabama, and the north central part of Florida and in Louisiana. Infection was apparently extending into the highlands of the Carolinas and into the northern part of Alabama. On the other hand the Mississippi delta reported considerably fewer malaria deaths in 1936. In 1938 the death rate was considerably lower in Georgia but higher in Alabama, Kentucky, Louisiana, Oklahoma and Tennessee. For the general distribution see map Fig. 7. While Tennessee is not one of the worst malarial states it has had for a number of years over 200 deaths annually from this disease. There are over 200 southern counties in which there has been a death rate of 25 or more per 100 000 between 1933–37. In 3 states—Arkansas, Florida and South Carolina—a large percentage of the population lives in the severely afflicted counties.

The figures show that there was a higher average malaria mortality observed in the southern United States in 1935–36 than there was 10 years before with malaria extending into areas in which this disease was then of little importance. The factors influencing this trend and the cyclic fluctuations of malaria mortality in the United States are not known. Malaria has recently become an increasing menace to life and to economic development in certain sections of the southern states. However recently there have been increased efforts to control the disease and while it has not yet been adequately attacked in some localities the most recent reports show a gradual decline in many areas. Nichols (1943) shows that since 1913 there has been a continued decrease in mortality. In 1940 it was 1.1 per hundred thousand.

# MALARIA

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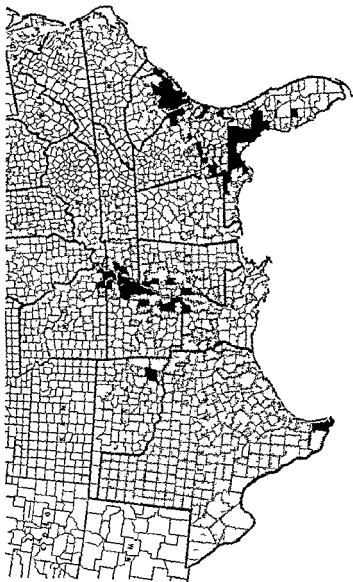


FIG. 2.—Malaria mortality in the United States after 1900 (Courtesy Southern Medical Journal)

The transmission of malaria north of the latitude of the Ohio River in the United States is today unusual. However Butts (1937) observed an outbreak of 120 cases in Camden County N. J. all of the cases being due to infections with *P. vivax*. Between 1912 and 1934 there had been a total of only 61 cases of malaria reported in Camden County.

*Tropical Regions*—Malaria has been so generally reported from all inhabited tropical areas that it would seem worth while to mention only a few of the very highly malarious sections. India already has been considered. In Africa many areas in the north are very malarious and it is very prevalent in West Africa and in Senegal and the Belgian Congo where malignant tertian rates often run up beyond 90 per cent the percentage of infected children being usually especially high. In Liberia Shattuck the writer and their associates in 1928, found in a single microscopical examination the rate of infection as high as 86 per cent.

In 49 boys examined up to 7 times in Sierra Leone by McDonald 98 per cent showed malarial parasites. In Nyasaland it has been estimated that from 4 to 9 out of every 10 children die before they are 6 years old largely because of malaria.

The southern geographical extension of malaria as intimated is far more limited than that in the northern hemisphere. It exists as far south as North Queensland in Australia and has occurred though rarely in South Queensland at 20 S. In South Africa it extends to Natal at 30 S. while in South America it reaches to 40 S. or the southern part of Argentina (Bahia Blanco).

*South America*—Only a few of the worst malarial centers in South America will be referred to. In Brazil malaria is the most prevalent and most serious disease of Amazonia. Different investigations along the Rio Negro have revealed that almost every inhabitant shows signs either of acute or chronic malarial infection. Souza Araujo states that in the city of Para in 10 months of 6909 examinations of blood 3140 or 45.4 per cent were positive for malaria. In further studies

*Plasmodium vivax* was found in 1645 *Plasmodium falciparum* in 1700 and *Plasmodium malariae* in 17. The proportion of malignant tertian (*Plasmodium falciparum*) was 54 per cent and of benign tertian (*Plasmodium vivax*) 46 per cent. He points out that malaria is much more serious in Para than in the southern Brazilian States and that a large proportion of the population acquires the infection shortly after birth and dies of it in infancy or early childhood. He has rarely found an individual in this region who has not had or who does not have malaria. The evidences of chronic malarial infection may be seen in the fact that the individual is robbed of his physical ability as well as of his intelligence and that he becomes depressed, inactive and apathetic towards the struggle of life. The depopulation of many regions in Amazonia is largely due to the great mortality from malaria.

Chagas points out that the valley of the River Amazon is without doubt, the region of Brazil where malaria presents its most severe types and is most intense as the mean conditions of temperature and of atmospheric humidity are optimum for the exogenous evolution of the plasmodium determining the high infecting power of the transmitter.

Uchoa in the Report of the National Department of Public Health of Brazil gives figures which obviously imply that very few days ever pass in the city of Manaus without at least one death from malaria although there is evidently no difficulty in obtaining quinine. Araujo Lima also states that in the suburbs about Manaus practically all of the inhabitants are chronically impaledated.

The lower Rio Branco is probably one of the worst malarial regions in the world and it was in this region that a member of one of our expeditions Dr. Theodore Koch Grunberg the eminent anthropologist lost his life.

from malaria. On the other hand the territory of the upper Rio Branco as well as all the mountainous areas are fairly healthy regions. All through the regions of the Rio Negro and lower Rio Branco there are unusual opportunities for the breeding of *Anopheles*, of which the moro coca (formerly reported as *Anopheles tarsimaculatus*) is the most common species. Hence it is evident why malaria prevails to such an extent.

DAVIS (1934) on the examination of 29,593 specimens of livers from persons dying in central or northern Brazil found the highest rates of malarial infection occurred in the states of Para, Amazona and Bahia in the order given. The lowest rate was in Ceara. In 1938 in certain areas in Brazil where the infection was spread by the African *Anopheles gambiae* 90 per cent of the population was found infected with a 10 per cent mortality.

In British Guiana according to the report of its Surgeon General in 1936 malaria also continues to be the most important disease in the Colony not only from the standpoint of causing the highest number of deaths but because of its crippling effects on the community. The majority of the inhabitants preserve a high degree of apathy towards it and its prevention. Year by year they suffer from repeated attacks since very few will undertake energetic treatment and still fewer voluntarily attempt any preventive measures in their dwelling or on their own land.

It is interesting to note that in Panama where such extensive anti-malarial public health work has been in progress for many years Callender and Gentzkow (1938) and Simmons (1939) report that the malaria incidence in the U.S. troops at Panama is the highest in the Army and over three times that of the employees of the Panama Canal. Also that it has not decreased appreciably in the past decade. This high rate is apparently due to the exposure of men in unsanitated areas during military operations.

During the year there were 525 primary malaria cases in a body of soldiers whose mean annual strength was 13,318 a rate per thousand per annum of 39.4. The rate for malaria in all Canal employees for the same period was 12.0. *Anopheles albimanus* is undoubtedly the most important malarial vector in Panama as it has recently been reported to be in other parts of Central America notably Guatemala, Puerto Rico and elsewhere. However Simmons points out that *A. p. clisimacula* is also an important vector in the Canal Zone especially in unsanitated areas.

Clark and Komp (1938) in the seventh year of their observations in the unsanitated area of the Panama Republic where nevertheless treatment had been given in previous years found that 29.0 per cent of 841 native adults and 41.4 per cent of 10,057 children harbored parasite. During 1936-37 which was a year of low malarial incidence in 682 positive examinations *falciparum* was present in 73 per cent, *tertiana* in 13.9 per cent and *plasmodia* in 1.5 per cent. Mixed infections varied from 0.7 to 9.5 per cent.

It is an interesting fact from the epidemiological viewpoint that Tahiti, Hawaii, Fiji, the Gilbert and Ellice Islands, Samoa, the Leeward Islands and Marquesas in the Pacific are malaria free due to absence of anophelines while certain other islands of the South Pacific as the Solomons and New Hebrides are badly infected. Granville Edge (1937) reports that the Society Islands and Rodriguez Island are also free of endemic malaria and anophelines. Mauritius was for a long period free from malaria but with the introduction of anophelines it became a malarial center. Brooks (1939) reports that in Mauritius the total number of patients admitted to hospitals with malaria was 3158 in an increase of 110 over the previous year. The case mortality was 3.86 per cent. Barbados was also formerly regarded as malaria free but in 1927 malaria broke out in the island apparently through the introduction of *A. albimanus* in the holds of trading ships and through the importation of laborers from Cuba. There was a serious epidemic of malignant tertian malaria which was at first reported as typhoid fever. The



initial cases showing vomiting and sometimes early jaundice but no rigors and no splenic enlargement. Manson Bahr (1940) reports that *A. a. bimaculatus* has now been largely exterminated in Barbados and Edge (1937) also reports Barbados free of anophelines and malaria. Ascension and St. Helena have also been said to be Malaria free Islands. In regard to the presence or absence of Malaria and Anophelines in New Caledonia authorities differ. Buxton (1927) and more recently Mumford (1942) have studied and reported upon the distribution of Malaria in the Pacific Islands and Mumford has pointed out the danger of the spread of the disease from infected Islands during the present World War.

### ETIOLOGY AND EPIDEMIOLOGY

**Etymology**—The malarial parasites of man are classified in the order Haemosporidia of the class Sporozoa and of the phylum Protozoa. The systematic position of the Sporozoa as well as of the other classes of Protozoa infecting man is shown in the following table.

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		Entamoeba	<i>E. bütschlii</i> <i>E. fragilis</i>
		Trypanosoma	<i>T. gambiense</i> <i>T. rhodensense</i> <i>T. cruzi</i>
		Leishmania	<i>L. donovani</i> <i>L. infantum</i> <i>L. braziliensis</i> <i>L. tropica</i>
II Flagellata (Mastigophora) Move by means of undulating membranes or flagellum multiply by division of the body longitudinally into two	Monozoa	Trichomonas	<i>T. hominis</i> <i>T. vaginalis</i>
		Chlamydomonas	<i>C. mesnili</i>
		Embryomonas	<i>E. intestinalis</i>
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III Infusoria (Ciliata) Move by means of numerous fine cilia and glide about swiftly multiply by transverse division of the body into two and also produce resistant cysts	Diplozoa	Giardia	<i>G. lamblia</i>
IV Sporozoa These have no motor organs. They live parasitically in the cells or tissues of other animals. Reproduction by spores	Heterotrichida	Balantidium	<i>B. coli</i>
		Nyctotherus	<i>N. faba</i>
	Coccidida	Eimeria	<i>E. stiedae</i>
		Isospora	<i>I. hominis</i> <i>I. vivax</i>
	Haemosporidia	Plasmodium	<i>P. malariae</i> <i>P. falciparum</i> <i>P. ovale</i> <i>P. knowlesi</i>
	Sarcosporidia	Sarcocystis	<i>S. tenella</i>

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**Order Haemosporidia**—Gametes unequal Sexual and asexual generations in unrelated hosts (arthropod vertebrate) Resistant spores usually absent Parasitic within blood cells

### HAEMOSPORIDIA

The order Haemosporidia includes the following genera *Plasmodium* *Haemoproteus* (*Halleridium*) *Leucocytozoon* *Babesia* *Theileria* and *Haemogregarina* The malarial parasites are the only Haemosporidia known to cause disease in man

*Plasmodium* (Laveran 1880)—This genus is characterized by the invasion of red cells by the parasite within which both schizogony and gametocyte formation take place by amoeboid movements by the production of pigment and by the extrusion of flagellum like processes (the microgametes) from the male sporont which occurs after the blood is taken from the animal host and allowed to cool.

Some consider the malarial parasites as belonging to 2 genera *Plasmodium* characterized by round sexual forms and including *P. vivax* and *P. malariae* and *Laverania* characterized by crescent shaped sexual forms and including but 1 species *L. malaris* (*P. falciparum*) that of aestivo autumnal malaria At present most protozoologists hold that a difference in the shape of the gametocytes is not sufficient for a generic distinction particularly in view of the fact that both the asexual and sexual life cycle are similar in all 3 species

It is believed that the 3 common human species of malarial parasites can only exist in man as an intermediate host and in certain species of *Anopheles* mosquitoes as definitive hosts It is the general belief also that mosquitoes of other genera are incapable of being infected with the *Plasmodium* causing human malaria and of transmitting them to man Nevertheless Williamson and Zane (1937) have succeeded in infecting *Culex bitaeniorhynchus* with *P. vivax* and *P. falciparum* and doubtfully with *P. malariae* While transmission of malaria with this presumptive culicine host has not been accomplished sporozoites in the salivary gland were demonstrated

**Malarial Infection in Animals**—Infection with related species of *Plasmodium* has been demonstrated in other mammals especially monkeys and in birds The following species have been identified in monkeys *P. reichenowi* resembling *P. falciparum* in chimpanzees and gorillas *P. kochi* in African monkeys *P. vivax* and *P. kneri* in macacus monkeys in Borneo and *P. pitheci* in the orang-outang (but not macacus) in Borneo all these resembling *P. vivax* *P. basileum* resembling *P. malariae* in many Central and South American monkeys and *P. rodhoni* in chimpanzees in Africa

A number of species of *Plasmodium* have been definitely established for birds Mosquitoes of the tribe *Culicini* (*Culex* mosquitoes) serve as definitive hosts in the case of the malarial parasites of birds The first demonstration of the mosquito life cycle of the malarial parasite (Ross 1898) was in the case of *P. praecox* of birds Grassi and Bignami then proved the occurrence of a similar cycle for the organism of human malaria in *Anopheles* mosquitoes

*Haemoproteus* and *Leucocytozoon* differ from *Plasmodium* chiefly in that schizogony occurs in the endothelial cells of the capillaries The gametocytes develop in the red cells producing pigment and eflagellating like *Plasmodium* and they undergo a similar cycle of development in the insect vector These parasites occur in birds and some cold blooded vertebrates When full grown the gametocytes are looped about the nucleus of the red cell like a halter The first observation of fertilization of the macro gamete by the flagellum (MacCallum 1897) was in the case of a *Haemoproteus* infection of crows

### Relationship between Human Plasmodia and Those of Other Animals

The fact that malaria is sometimes contracted in regions apparently uninhabited by man has led certain investigators to believe that some of the lower animals may harbor the human plasmodia and act as reservoirs of infection for man It has even been suggested that the chimpanzee may be in some instances susceptible to *P. vivax* and *P. falciparum* infec

initial cases showing vomiting and sometimes early jaundice but no rigors and no splenic enlargement. Manson Bahr (1940) reports that *A. albimanus* has now been largely exterminated in Barbados and Edge (1937) also reports Barbados free of anopheles and malaria. Ascension and St. Helena have also been said to be Malaria free Islands. In regard to the presence or absence of Malaria and Anopheles in New Caledonia authorities differ. Buxton (1927) and more recently Mumford (1942) have studied and reported upon the distribution of Malaria in the Pacific Islands and Mumford has pointed out the danger of the spread of the disease from infected Islands during the present World War.

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from a pathological standpoint is to the effect that they are not identical the disease produced in man by the species from monkeys being usually of a much milder nature

**Life History of the Malarial Parasite**—All of the parasites belonging to the genus *Plasmodium* have an asexual and a sexual cycle of development. The first known as the endogenous cycle is passed in man and the process of reproduction during this cycle is called schizogony. The second or sexual cycle known as the exogenous cycle is passed in some species of mosquito (though certain preliminary stages occur in the blood) the process of reproduction during this cycle being called sporogony. In the blood of man the plasmodia live within the red blood corpuscles and eventually destroy many of these cells. In addition to the natural method of infection by the bite of the mosquito man may also be infected by the intravenous or subcutaneous injection of blood containing the parasite.

**CYCLE IN MAN 1 Asexual Cycle (Schizogony)**—In the normal transmission of malaria in man an infected *Anopheles* mosquito at the time of feeding on the human blood introduces through a minute channel in its hypopharynx the infecting sporozoite of the sexual cycle. The sporozoite is a slender slightly curved organism measuring from about 12-15 $\mu$  in length. It tapers at both ends, has an elongated nucleus and is devoid of pigment. (Missiroli Knowles and DeMeillon (1936) have observed nuclear division and sometimes multiple nuclei in sporozoites in the mosquito.) It is capable of slight undulatory movement. This falciform sporozoite after a variable period of time enters the red cells, assumes a rounded shape and is then known as a trophozoite.

It has generally been assumed partly on the basis of an observation by Schaudinn that after entering the blood vessel the sporozoite quickly penetrates a red cell. Some investigators still incline to this belief and think that the period of incubation of malaria (often from 10-14 days) depends upon the time necessary for the multiplication of a sufficient number of parasites in the blood to produce symptoms. However there is a large amount of evidence that following natural inoculations of man by sporozoites the parasites are not demonstrable in the circulation until after the lapse of at least several days. Because treatment of a patient with quinine during the first 5 days after the bite of the infected mosquito does not apparently reach and destroy the sporozoites and the sporozoites are not visible in the blood or corpuscles during this period it has been suggested that they do not at once enter the red blood corpuscles.

Boyd and Stratman Thomas (1934) found that after heavy experimental infection with *P. vivax* by bites of 15 infected mosquitoes the blood did not become infectious for a second individual by transfusion until the ninth day, parasites being found in films on the eleventh day. Boyd and Mathews (1930) also found that after heavy experimental infection of another patient a Negro (#18) with *P. falciparum* his blood did not become infectious for another individual (#1863) by transfusion until about the 8th day, parasites being found in the blood (of #1818) in films on the 10th day. This suggests that the sporozoites may undergo a preliminary period of development in the tissues before invading the blood cells and beginning active multiplication in them.

tion Some of the species of malaria plasmodia occurring in monkeys are similar in morphology to the human species, and malaria in monkeys may be transmitted by anopheline mosquitoes Nevertheless, it has not been demonstrated that any of the parasites of man are identical with any of the plasmodia of monkeys

Reichenow (1937) who described 3 species of plasmodia of chimpanzees in the Cameroons considered them to be identical with the human species *vivax falciparum* and *malariae* However attempts in earlier years to transfer the human species to any of the lower animals have failed Single exceptions have been reported by Mesul and Roubaud (1920) who claimed to have infected a chimpanzee with *P. vivax* while the Tahaferros (1934) reported transmitting *P. falciparum* to the howler monkey (*Alouatta* species) in Panama The infection was transmitted from 9 human beings to 9 monkeys and sub inoculated from one monkey to another

On the other hand Gonder and Rodenwaldt and Blacklock and Adler all were unable to infect human beings by subcutaneous and intravenous inoculations of blood from a chimpanzee infected with *P. kochi* Rodhain and Muyile (1938) have attempted to infect 3 patients requiring malaria therapy with the *vivax* and *falciparum* type of the parasites of chimpanzees but also without success none of the patients contracting the infection More recently Rodhain (1940) inoculated the blood of a chimpanzee harboring *P. rodhaini* (resembling *P. malariae*) into a human being suffering with general paralysis Forty days later an attack of fever occurred with the malarial parasites present in blood films

However since the discovery of *Plasmodium knowlesi* and the first successful inoculation of this strain of monkey malaria to 3 human volunteers by Knowles and Das Gupta in 1932 numerous other successful inoculations of this parasite into man have been made Van Rooyen and Pile (1935) in England Ciuca (1937) in Rumania and Milam and Kusch in New York (1938) have successfully inoculated this parasite into man in the treatment of numerous cases of general paresis Milam and Kusch found that susceptibility appeared to be universal from studies of 29 white persons but in 6 negroes there was almost no response

Milam and Coggeshall (1938) have also found negro patients less susceptible to *P. knowlesi* than white patients hence resistance to infection with this parasite is evidently a racial factor The infection of man with this parasite often terminates without treatment

The clinical course of *P. knowlesi* malaria in man very closely resembles that of *P. vivax* malaria the outstanding point being the shorter term of the former The number of paroxysms per patient in Milam and Kusch's series varied from 2 to 15 averaging 10 Only about one half of the patients experienced one or more definite chills However in several cases it was necessary to administer quinine to the patients on account of the severity of the clinical symptoms All of these however responded promptly to treatment with quinine

Rodhain (1936) has shown that African monkeys (species of *Cercopithecus* and *Papio*) are especially subject to infection with this parasite which may cause the death of these animals with symptoms of haemoglobinuria Infection in *Macacus rhesus* monkeys with *P. knowlesi* is invariably fatal unless treated

Ionesco Mihailesti (1934) and his associates have also reported successful infection of 3 human volunteers with *P. inui* obtained from a baboon dying of this infection

Hence it seems evident that some of the parasites of anthropoid apes and monkeys are closely related to those of man even though the evidence

is a tendency for the parasites to develop at nearly the same rate and to fall gradually into (one or) two groups, all members of which show about the same stage of development. Sporulation of all the parasites in the group thus occurs at about the same time. The onset of the malarial paroxysm corresponds to the time of sporulation and is attributed to the sudden liberation into the blood of toxic substances from the disintegrated red cells.

2. **Sexual Cycle**—Sexual forms (gametocytes) appear in the blood at varying intervals after fever has started, perhaps because conditions become less favorable for con-

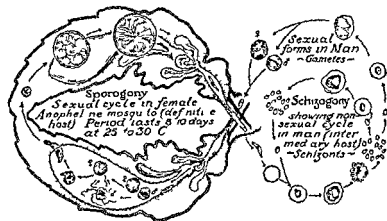


FIG. 3.—Sexual (sporogony in mosquito) and nonsexual (schizogony in man) cycle of the malarial parasite. The sporogony diagram at the left shows in lower portion the fertilization of the female gamete by the microgamete. The vertical line stage of the zygote shown passing into the wall of the mosquito's stomach later to become the non-mature zygote packed with sporozoites as shown in the upper diagram of the developmental process in the mosquito's stomach.

tinued multiplication. They may be present from the first in benign tertian after a week in aestivo autumnal and may appear only after several months in quartan. They probably develop from pre-existing asexual parasite. The period of growth is about double that of the schizonts but they probably do not become mature and infective until after a week or ten days. The life of a gametocyte has been estimated at from 30 to 60 days. The female macrogametocyte tends to be larger, stains more deeply blue, and contains more pigment but less chromatin than the male microgametocyte.

**Sexual Cycle in the Mosquito (Sporogony)**—When blood containing gametocytes is taken into the stomach of a suitable *Anopheles* mosquito the microgametocyte undergoes a process of exflagellation. This sometimes occurs and can be observed in fresh moist blood films at room temperature. The pigment shows very active movement. Long slender bulbous-tipped flagellum-like processes are gradually extruded (the microgametes). These show active lashing movement and finally break away and swim about until they find a macrogametocyte. The latter after undergoing a nuclear reduction with the formation of polar bodies becomes a macrogamete. It is

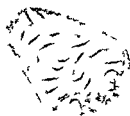


FIG. 4.—Sporozoites (After Graess)

James conceived the idea that the sporozoites injected by the mosquito are immediately carried to the visceral circulation where they are engulfed by the cells of the reticulo-endothelial system. Here they multiply and produce stages which are able to enter the red blood corpuscles and thus reach the peripheral circulation. In order to prove this hypothesis, several species of avian malarial parasites were studied in birds. The observations of James and Tate in England, of Raffaele in Italy, of Huff and Bloom and Warren and Coggeshall in the United States, of Kikuth in Germany and of Brumpt in France have shown that during the latter part of the so-called period of non infectivity of the blood (in which no parasites are visible by the microscope in the blood) birds nevertheless can be infected by inoculation with a small portion of the spleen, liver or brain, thus supporting the idea that the reticulo-endothelial system is parasitized during the prepatent period.

However it has not yet been conclusively demonstrated that the sporozoite injected by the mosquito into the avian host is the immediate antecedent of the parasite in the reticulo-endothelial cells. Nor is it yet permissible to conclude that the asexual phases in the malarial parasite of the bird are exactly parallel to those which occur in the plasmodia of man.

Thus Missiroli reports that the sporozoites of *P. praecox* leave the point of inoculation in canaries through the lymphatic vessels within 1 to 5 minutes. However Boyd and Kitchen (1939) in a study for the demonstration of sporozoites in human tissue found 24 hours after the biting of numbers of infected mosquitoes several sporozoites in one lymph node excised. These however were always in connective tissue and never in lymphoid tissue. The sporozoites were unaltered in appearance. They remark that their experiments do not suggest that the lymph passages are the route by which the main proportion of sporozoites reach their destination.

The exo-erythrocytic schizogony so carefully studied for some species of plasmodia in birds has not as yet been observed in human or even simian malarial infection. However some of the forms seen in avian malaria have been reported in infections with *P. mearnsi* and *P. falciparum* in which larger non pigmented schizonts were found in endothelial cells. Huff and Porter (1940) have completely reviewed the literature on the subject to which the reader is referred for further information.

Huff et al (1943) suggests the term cryptozoite for the first generation of malarial parasite developing from a sporozoite. This stage is exoerythrocytic and may be a uninnucleate form, a multinucleate form or a schizont. If the parasite enters directly into the red corpuscle there would be no cryptozoites in the cycle of such a species.

In any case the sporozoite after an interval enters a red cell. It then assumes a somewhat round or irregular shape becoming a trophozoite, and progressively enlarges forming from the disintegrated haemoglobin many fine granules of pigment haemozoin formerly regarded as melanin. When its nucleus begins to develop it is known as a schizont. As it approaches maturity (after 48-72 hours according to the species) it fills the greater part of the cell. The pigment which has been scattered becomes clustered in the center of the parasite. The nuclear chromatin of the schizont which has been relatively compact, divides into from 8-32 minute particles which become scattered through the cytoplasm. About these as centers the cytoplasm of the parasite divides into small spore like bodies (merozoites). The organism now termed a merozoite then ruptures liberating the merozoites into the plasma. These seek out and penetrate fresh red cells and the cycle is repeated while the residual body of the merozoite with the contained pigment is ingested by free endothelial cells of the blood vessels or by wandering phagocytes usually large mononuclears (pigmented leucocytes).

Multiplication in geometric progression goes on until after about two weeks (incubation period) a sufficient number of parasites has been produced to cause clinical symptoms. It is estimated that there must be at least 200 per cmm or about a billion in the entire body. As a rule there

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There are certain questions connected with the life history of the malarial parasite in man which are of interest

1 *Intra or Extracellular Location*—It is usual to consider the parasite as developing within a red cell and in this position destroying the red cell Rowley Lawson however has maintained that the parasites are exclusively extra-cellular and that they adhere to the red cells by loop-like pseudopodia which encircle a portion of the red cells and digest the haemoglobin of such an area However this view is not generally accepted

2 *Malarial Toxin*—The exact nature of the toxic material liberated at the time of the paroxysm is not known Rosenau's experiments tend to show that a fever producing toxin was thrown off at this time The study of the pathology of malaria indicates the haemolytic nature of the toxin and it is active both in the blood and bone marrow Brown suggested that the pigment produced by the parasite in its metabolism of the haemoglobin of the red cell may act as a haemolysin he having found that intravenous injections of haematin were capable of producing marked anaemia It is well known that a far greater number of red cells are destroyed in a paroxysm than would be accounted for by the actual percentage of cells destroyed by parasites The endothelial cells take up actively this malarial pigment or haemozoin and may be damaged by it Haematin injections also tend to destroy leucocytes and platelets Abram regarded the paroxysm as an anaphylactic reaction precipitated by the liberation of the malarial (foreign) protein at sporulation This occurs some hours before the cold stage which he regards as a manifestation of anaphylactic shock A leukopenia and a lowering of the blood pressure preceding the paroxysm are considered evidence of a haemoclastic crisis.

3 *Transmission to Larvae of the Mosquito*—It has been suggested that the sporozoites might enter the ovaries and ova as well as the salivary glands of the mosquito so that a second generation of mosquitoes might transmit malaria The sporozoites circulate in the haemocoel which is the body cavity functioning as the blood vascular system in insects Hence they are sometimes found in all the tissues of the mosquito as well as in the salivary glands and ducts but there is no evidence whatever in favor of congenital transmission in the mosquito However after the sporozoites reach the salivary gland of the mosquito the insect may remain infective for long periods Mosquitoes have been found capable of transmitting malaria as long as 90 days after infection In some species of mosquitoes oöcysts may develop in the stomach wall but further development of the parasite does not occur

4 *Congenital Malaria*—There has been some question as to the possibility of congenital malaria Heiser has reported a case of an infant which showed crescents in its blood by the end of one week from birth The mother showed the same infection and it was thought the child must have been infected through the placental circulation Clark in numerous examinations of the blood of the new born failed to find infection even when the mother's blood teemed with parasites In one case where the child showed infection shortly after birth there had been an accident to the placenta and he believes that instances of so called congenital malaria may be explained in this way In malarial infection at the time of parturition massive infection of the placenta is common even when there are few parasites visible in the peripheral circulation

Garhnan (1938) made observations regarding the presence of malarial infections of the placenta in 500 cases of pregnancy in native women in Kenya and in a long series of observations on foetal bloods in cases of heavy placental infections and was unable to demonstrate in a single instance the passage of parasites from mother to foetus

Blacklock and Gordon in Sierra Leone found that 36 per cent of pregnant women infected with *P. falciparum* showed intensive infection of the placenta leading to death of the foetus Jean and Nitson have reported 8 instances of children either born dead or dying soon after birth in 6 of whom malarial parasites were found in the spleen



then penetrated by a single microgamete which fuses with the nucleus forming the zygote. The zygote (called at this stage a vermiculus or ookinete) elongates and becomes



FIG. 5.—Digestive tract of *Anopheles* the stomach of which is covered with numerous zygotes or oocysts of *Plasmodium falciparum*. a midgut malpighian tubules oocysts stomach b sucking bladders or pumping organ c salivary gland (MacNeal from Doflein modified after Koss and Grassi.)

capable of worm like movement which enables it to bore through the wall of the mosquito's stomach. It stops just under the delicate outer layer where after 3 to 4 days it becomes encapsulated to form an oocyst. The latter grows into a rounded wart like protuberance 50  $\mu$  in diameter on the outer surface of the stomach wall. Its contents now undergo important changes. The nucleus divides repeatedly and a number of faintly outlined cells are formed varying in size and number which are called sporoblasts. By further subdivision of the nuclear chromatin and subsequently of the cytoplasm there are formed great numbers of minute falciform *sporozoites*. The number may vary from several hundred to 1000 in a single cyst and there may be 500 cysts in the stomach of a single mosquito. When development is complete the cyst ruptures and trees the sporozoites in the body cavity or hemocoel of the mosquito. Many of these migrate into the salivary glands and thence by way of the venous salivary duct in the hypopharynx they are introduced into the circulation of the person bitten by the mosquito and start an asexual cycle. The mosquito is thus the definitive host and man is the intermediate host. The mosquito suffers no evident injury from the infection. Usually its life is not shortened nor is its fertility lowered.

Russell (1930) has found that it is possible to stain malarial oocysts in living mosquitoes by feeding them on a 10 per cent glucose solution to which has been added a small quantity of eosin (water soluble). In mosquitoes which feed on this solution for 2 days the oocysts are clearly stained and so more readily seen on dissection of the mid gut. Sporozoites are not stained with eosin.

Ross noted dark brown or black bodies in the oocysts of some mosquitoes which have been described as Ross's black spores. Their nature has been controversial. It is probable that the masses in some instances are degeneration products of the oocysts contents though sometimes thickening of the tracheal tubes of the mosquito resemble them and they have been found in mosquitoes not infected with malaria. Brumpt (1938) suggests that the black spores may be due to the chitinization of the contents of the oocysts. In the study of a lot of mosquitoes he found them only in those that had fed on infected fowls and never in those fed on uninfected fowls. He thinks they are evidence that infection of the mosquito has occurred.

The duration of the life cycle in the mosquito varies with the temperature and the species of parasite. Below 18 C little or no development occurs but the parasites in the mosquito may survive ice box temperatures and even freezing (Coggeshall 1939). Once sporozoites have developed a mosquito may remain infectious for three

months and may infect many individuals since only a part of the parasites are discharged with each bite.

Plate II illustrates in a schematic way the evolution of the three common malarial parasites of man while in the frontispiece Plate I,

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2 *Malarial Toxin*—The exact nature of the toxic material liberated at the time of the paroxysm is not known Rosenau's experiments tend to show that a fever producing toxin was thrown off at this time The study of the pathology of malaria indicates the haemolytic nature of the toxin and it is active both in the blood and bone marrow Brown suggested that the pigment produced by the parasite in its metabolism of the haemoglobin of the red cell may act as a haemolysin he having found that intravenous injections of haematin were capable of producing marked anaemia It is well known that a far greater number of red cells are destroyed in a paroxysm than would be accounted for by the actual percentage of cells destroyed by parasites The endothelial cells take up actively this malarial pigment or haemozoin and may be damaged by it Haematin injections also tend to destroy leucocytes and platelets Abrami regarded the paroxysms as an anaphylactic reaction precipitated by the liberation of the malarial (foreign) protein at sporulation This occurs some hours before the cold stage which he regards as a manifestation of anaphylactic shock A leukopenia and a lowering of the blood pressure preceding the paroxysm are considered evidences of a haemoclastic crisis

3 *Transmission to La vae of the Mosquito*—It has been suggested that the sporozoites might enter the ovaries and ova as well as the salivary glands of the mosquito so that a second generation of mosquitoes might transmit malaria The sporozoites circulate in the haemocoel which is the body cavity functioning as the blood vascular system in insects Hence they are sometimes found in all the tissues of the mosquito as well as in the salivary glands and ducts but there is no evidence whatever in favor of congenital transmission in the mosquito However after the sporozoites reach the salivary gland of the mosquito the insect may remain infective for long periods Mosquitoes have been found capable of transmitting malaria as long as 90 days after infection In some species of mosquitoes oöcysts may develop in the stomach wall but further development of the parasite does not occur

4 *Congenital Malaria*—There has been some question as to the possibility of congenital malaria Heiser has reported a case of an infant which showed crescents in its blood by the end of one week from birth The mother showed the same infection and it was thought the child must have been infected through the placental circulation Clark in numerous examinations of the blood of the new born failed to find infection even when the mother's blood teemed with parasites In one case where the child showed infection shortly after birth there had been an accident to the placenta and he believes that instances of so called congenital malaria may be explained in this way In malarial infection at the time of parturition massive infection of the placenta is common even when there are few parasites visible in the peripheral circulation

Garhnan (1938) made observations regarding the presence of malarial infections of the placenta in 500 cases of pregnancy in native women in Kenya and in a long series of observations on foetal bloods in cases of heavy placental infections and was unable to demonstrate in a single instance the passage of parasites from mother to foetus

Blacklock and Gordon in Sierra Leone found that 35 per cent of pregnant women infected with *P. falciparum* showed intensive infection of the placenta leading to death of the foetus Jean and Nitson have reported 8 instances of children either born dead or dying soon after birth in 6 of whom malarial parasites were found in the spleen

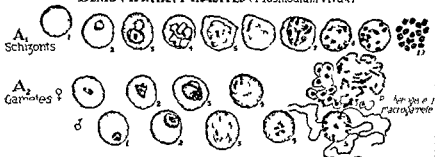
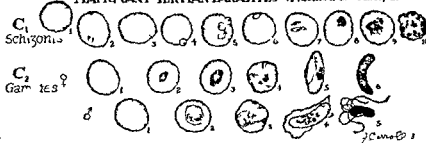
BENIGN TERTIAN PARASITES (*Plasmodium vivax*)QUARTAN PARASITES (*Plasmodium malarie*)MALIGNANT TERTIAN PARASITES (*Plasmodium falciparum*)

PLATE II

More recently James and Brown and Tanner and Hewlett have reported undoubted cases of congenital transmission of benign tertian malaria occurring in London 2 months after birth. Meacham (1938) has reported another congenital case in which the peripheral blood of the mother was completely clear of parasites while the placenta showed a heavy infection. The child's spleen was palpable on the 7th day. On the 17th day tertian rings, schizonts and gametocytes were present in the child's blood.

Green (1938) reports a woman at full term admitted to the hospital with severe malaria. The child was born after an easy labor of 24 hours. The placenta appeared normal. No parasites were seen in the baby's blood. The following day the infant died in coma with hyperreflexia. On postmortem examination the brain was found to be congested and 4 parasites per field were seen in smears of blood of the cerebral veins.

Colten (Lancet January 29 1944) has reported six additional cases.

## DESCRIPTION OF PLATE II OF MALARIAL PARASITES

### Cycle of Development of the Malarial Parasites (Schematic)

#### Benign Tertian Parasites

A1 *Trophozoites* and *Schizonts* 1 Normal red cell. 2 Young ring form. 3 Amoeboid or figure-of-eight form showing Schuffner's dots. 4 Amoeboid form showing increased chromatin (24 to 30 hours). 5 Segmentation of nucleus. 6 Nuclear halves further apart, red cells enlarged and pale. 7 Further division of nucleus. 8 Unusual division form. 9 Typical merocyte. 10 Rupture of merocyte liberating merozoites.

A2 *Female gametes* 1 Young form showing solid instead of ring form staining. 2 Half grown form. 3 Rapidly growing form with compact nucleus and clear vacuolated zone. 4 Full grown macrogametocyte showing eccentrically placed chromatin and much pigment in deep blue stained protoplasm. *Male gametes* 1 Young form similar to female one. 2 Half grown form showing central chromatin. 3 Full grown microgametocyte showing large amount of centrally placed chromatin with light blue protoplasm surrounding. 4 Division of chromatin occurring in microgametocyte and developing in wet preparation. NOTE—Chromatin division in gametocytes does not take place until blood is withdrawn. 5 Spermatozoon like microgametes developing from the microgametocyte. This only occurs in wet preparations or in the stomach of the mosquito. Parthenogenetic macrogamete this object supposed by Schaudinn to be a parthenogenetic female has since been interpreted as two parasites (schizont and macrogamete) in a single red cell.

#### Quartan Parasites

B1 *Trophozoites* and *Schizonts* 1 Normal red cell. 2 Young ring form. 3 Older ring form. 4 Narrow equatorial band. 5 Typical band form. 6 Oval form showing division of chromatin. 7 Early stage merocyte. 8 Daisy form merocyte.

B2 *Male gametes* 1 Young solid form. 2 3 4 Developmental stages microgametocytes. 5 Flagellated body in wet preparation showing microgametes developing from microgametocyte. *Female gametes* 1 Young oval form. 2 Somewhat older stage. 3 and 4 Mature macrogametocytes (same as benign tertian).

#### Malignant Tertian Parasites

C1 *Trophozoites* and *Schizonts* 1 Normal red cell. 2 3 4 5 6 Young ring forms. These are hair-like rings and are the only forms besides crescents to be found in the peripheral blood. In very heavy infections or in smears from spleen the following forms are found. 7 Beginning division of chromatin. 8 and 9 Further division. 10 Merocyte.

C2 *Female gametes* 1 and 2 Young macrogametocytes. 3 Older stage. 4 Development in red cell. 5 and 6 Fully developed female crescents showing clumping of pigment and rich blue color. *Male gametes* 1 and 2 Developing forms. 3 and 4 Fully developed microgametocytes. 5 Flagellated body developed in wet preparation.

In the second case the woman had 2 rigors during labor. Malarial parasites were found in the blood. The child was born cyanosed and comatose. No parasites were found in the blood from the cord and the placenta appeared normal. However the infant's blood contained parasites. In the third case in an infant 2 days old after normal labor parasites were also found in the blood.

Das Gupta (1939) found mature schizonts in the blood of an infant 15 hours after birth which proved to him beyond doubt that the infection was acquired *in utero* at least 33 hours before birth. He points out that congenital malaria while rare does occur when there is a failure of the protective effect of the placenta. The protective action fails when there is any injury during pregnancy resulting in placental haemorrhage. He studied the question experimentally in a pregnant rhesus monkey infected with *P. knowlesi*. He found that the foetus was entirely free from parasitic infection even when the maternal sinuses of the placenta were crammed with parasitized red cells more than 95 per cent being infected. He suggests that the duration of the infection may perhaps be responsible for the failure of the protective function of the placenta at times.

5. *Cultivation of Parasite*—The cultivation of the malarial parasite *in vitro* was first reported by Bass in 1911 in defibrinated blood containing glucose solution. The brothers Thompson later confirmed this work. Asexual multiplication of the parasites has been reported in the 3 common types of parasites and in the case of the subtertian as many as 4 successive generations were obtained. Sinton has since reported the cultivation of crescents in artificial culture of blood after 10 days incubation. No one has yet been able to maintain the plasmodia *in vitro* for more than a few generations. It has been reported that the growing parasites of *P. falciparum* show remarkable tendency to clump together which is not observed in *P. vivax*.

Boyd (1939) reports that in his laboratories from the technical standpoint the *in vitro* cultivation or extracorporeal preservation of the parasites has not been of practical value.

## HUMAN SPECIES OF PARASITES

As before stated, there are 3 common species of malarial parasites of man (1) *Plasmodium vivax*, that of benign tertian—cycle 48 hours (2) *Plasmodium malariae* that of quartan—cycle 72 hours and (3) *Plasmodium falciparum* that of aestivo autumnal or malignant tertian—cycle 48 hours. The rarer parasite *P. ovale* gives rise also to benign tertian attacks of fever, with a cycle of 48 hours.

*Plasmodium vivax* (Cycle 48 Hours)—The cause of benign tertian malaria is the most widespread of the 4 species occurring throughout the tropics and sub tropical regions as well as in extensive areas in the temperate zone. It has been reported as far north as southern Sweden, England and the Great Lakes of North America and as far south as Argentina and Australia.

*Morphology*—In fresh unstained preparations taken at the time of the paroxysm or shortly afterward the benign tertian schizont or nonsexual parasite is seen as a grayish white round or oval body whose outlines are differentiated with difficulty from the infected red cell. It is about one fifth of the diameter of the red cell and may be recognized by noting the amoeboid activity. In about 18 hours fine pigment particles appear and the parasite becomes more distinct. After 24 hours the lively motion of the pigment and the projection of pseudopod like processes in a pale and swollen red cell make recognition very easy. When about 30 to 36 hours old the amoeboid movement ceases. Approaching the macrocyte stage the pigment tends to clump into 1 or 2 pigment masses and one can recognize small oval highly refractile bodies within the sporulating parasite.

The gametocytes or sexual forms do not show amoeboid movement but the fully developed gametocyte which is generally larger than the red cells has abundant pigment which is actively motile in the male gametocyte and nonmotile in the female. The male gametocyte is more refractile is rarely larger than a red cell and shows yellow brown short rod like particles of pigment. About 15 minutes after the making of a fresh preparation these male gametocytes often throw out 4 to 8 long slender lashing processes which are about 15 to 20 microns long. These spermatozoon like bodies now break off from the parent cell and with a serpent like motion glide away in search of a female gamete (macrogamete) pushing the red cells about in their passage through the blood plasma. These are the microgametes. The female gametocyte is larger than a red cell is rather granular and has more abundant dark brown pigment than the male.

**Stained Preparations**—In dried films stained by some Romanowsky method as that of Wright Leishman or Giemsa we note small oval blue rings about one fifth



FIG 6—*Plasmodium* (Benign tertian) Development of schizonts in peripheral blood of man. Red cell swollen and tained feebly. Not Schüffner's dots.  $\times 2200$  (M. Neill after Dofle n)

of the diameter of the infected yellowish pink erythrocyte. One side of the ring is distinctly broader than the rather fine opposite end which seems to hold a round yellowish brown dot the chromatin dot and has a resemblance to a signet ring. These small tertian rings of the nonsexual parasites (schizont) are seen about the time of the commencement of the seating stage of the parovysm. Two chromatin dots in the line of the ring are rare as is also true of more than one ring in a red cell.

When the parasite is about 24 hours old we note that it contains much pigment and has an amoeboid or multiple figure of eight contour is about three fourths the size of a red cell and that the infected red cell is about one and one half times as large as in the beginning and presents a washed out appearance. It is an anaemic looking cell. We also note as characteristic of a benign tertian infection reddish yellow dots in the pale red cell which are known as Schüffner's dots. These have been regarded as characteristic for benign tertian but they are also reported in *P. ovale*.

A few hours before the completion of its 48 hour cycle the contained pigment begins to clump the chromatin to divide and finally we have a sporulating parasite in which the 16 to 20 small round bluish bodies with chromatin dots are irregularly distributed over the area of the merocyte.

The gametocytes or sexual parasites show a thicker blue ring and have the chromatin dot in the center of the ring. The pigmentation of the half grown gametocytes is more marked than that of schizonts of equal size. The shape of the gametocytes is not amoeboid as is that of the 24 to 36-hour-old schizont but round or oval. The fully grown gametocyte have the pigment distributed as the chromatin is a single aggregate on—just the opposite of nonsexual parasites. The male gametocyte stains a light grayish blue and has a very large amount of chromatin usually centrally placed. The



FIG 7—*Plasmodium malarium* Malarial schizont in malarial blood just before and at onset of chill.  $\times 2200$  (M. Neill after Dofle n)

female gametocyte stains a pure blue has only about one tenth as much chromatin as plasma with the chromatin often placed at one side. The pigment of the female gametocyte is dark brown while that of the male is yellowish brown.

**Plasmodium malariae**—The course of quartan malaria has a cycle of 72 hours. Quartan fever is relatively rare. Formerly it was reported as more a disease of the temperate zone than of the tropics. The disease, clinically, is the mildest of the 3 common types but it is sometimes very resistant to treatment and prone to late relapses. It has been reported frequently as a cause of malarial nephritis (MacFie, Giglioli, Lambers). In the localities in which it occurs usually it has shown a limited topographical distribution being found chiefly in scattered areas.

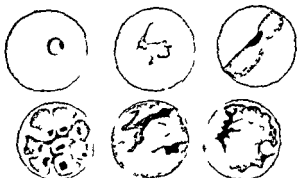


FIG. 8.—*Plasmodium malariae* (Quartan). Development of asexual parasite in blood of man.  $\times 2200$  (From MacNeal after Dofflein.)

It has been encountered especially in the Mediterranean regions and in India, Ceylon and the Malay Peninsula. In Africa it has been found in the central tropical belt, Kenya to Sierra Leone. In the western hemisphere it occurs in the southern United States, in Panama and Brazil. Chandler (1940) points out it was introduced into New Orleans, La. by drug addicts a few years ago and has now become endemic there. It is relatively rare in the West Indies but common in Antigua.

In Brazil, Souza Araujo found this parasite in less than 0.5 per cent of the malarial cases while Clark in Panama (1939) found it in approximately 5 per cent of the malarial infections. On the other hand, Manson Bahr (1936) notes that Sayes has observed in the western Solomon Islands as Wenyon has in Salonika that the quartan is the commonest type of malaria in children between 2 and 10 years. After that age it is seldom met with. This observation has not been borne out by experience in many other countries. However, Schwetz (1938) points out that in parts of the Belgian Congo quartan fever while hardly ever found among Europeans is frequent among natives and affects up to 50 per cent of native child parasite carriers. Gordon and Davey (1932) found that in Freetown, Sierra Leone there has been a rapid increase in quartan malaria since 1935 where the infection spread from a small section of the city over the entire community. In their last survey 68 per cent of the positive specimens revealed *P. malariae*. They were unable to infect with *P. malariae* either of the 2 common vectors of tertian and aestivo autumnal malaria in this region, namely *Anopheles gambiae* (costalis) and *A. funestus*. They suggest however that *A. gambiae* is the sole or chief vector of the quartan infection.

**Morphology**—In fresh preparations the young quartan schizont has only slight amoeboid movement in comparison to *P. vivax* and as development proceeds the rather darker brown and coarser pigment tends to arrange itself peripherally about the band-shaped or oval parasite and sometimes shows less oscillation than in *P. vivax*.

The infected red cell shows but little change. At the end of 72 hours the rather regular daisy form of the merocyte is often more distinct than that of the benign tertian merocyte.

The distinctions between the male and female gametocytes are similar to those of the benign tertian gametocytes. In Romanowsky stained smears it is difficult to distinguish the young quartan schizont from the benign tertian one but after 24 hours the tendency of the quartan schizont to assume equatorial band forms across a red cell of normal size and staining characteristics and without Schüffner's dots makes the differentiation clear. In the fully developed sporulating parasite or merocyte the (6-12) commonly 8 merozoites assume a regular distribution giving it a daisy appearance.

The gametocytes show practically the characteristics of the benign tertian ones but are smaller.

*Plasmodium falciparum* of aestivo autumnal or malignant tertian malaria has a cycle of development of about 48 hours. However this is variable and there is not as much tendency to simultaneous development of the parasites as is shown by the other 2 species. As a result sporulation is often protracted or continuous and the fever is irregularly remittent or continuous or the paroxysms if they occur are protracted.



FIG. 9.—*Plasmodium falciparum* (Malignant tertian). Nonsexual cycle blood and internal organs of man. Note multiple infections of single red cell. (F. M. MacNell after Doflen.)

*P. falciparum* is widespread throughout tropical and subtropical regions but is relatively rare in the temperate zone. It is the most dangerous type of malarial parasite, sometimes giving rise to pernicious attacks which may be quickly fatal. However (if reinfection is avoided) sometimes it dies out more quickly and is often more susceptible to treatment than the other species.

*Isophlogy*.—The young trophozoites are sometimes difficult to detect in fresh preparations appearing early in the hot stage of the attack as minute crater-like dots about one sixth the diameter of the red cell. However they show active amoeboid movement. They are usually in the periphery of the red cell. It is common to find several parasites in the same cell. They gradually enlarge to become about a third the diameter of the cell and occasionally a few fine brownish pigment granules may appear. At this stage the infected red cells disappear from the peripheral blood except in rare instances in very severe infections. However Joly (1936) in the French Congo reports that all stages in the development of schizonts and gametocytes of *P. falciparum*



may be seen in the peripheral blood of children below the ages of 4 or 5 year but not in older children

In severe infections there may be extraordinary numbers of parasites present many in every field and up to 8 in a single cell. It has been estimated that there may be three trillion in the entire body

In stained films the young parasites appear as hair like rings often with a chromatin dots on one side of the ring. They often appear as if plastered on the periphery of the cell or as if they had destroyed a rounded section of the rim of the cell. Rarely they may be bacillary in shape and show no vacuole being recognizable by the red chromatin dot. The infected cells may show diffuse basophilic staining and distortion. Schüffner's dots do not occur but in heavily stained films Maurer's dots may be seen as coarse scattered deep brick red dots or clefts. It is not possible to identify the species of parasite if the early ring forms alone are present. However if fine rings are present on one examination and if 12 hours later they show definite thickening *P. falciparum* can be excluded as this species nearly always disappears from the circulation by the time this stage is reached.

The infected red cells tend to agglutinate and to adhere to the walls of the capillaries of the internal organs in which they may form plugs and cause grave symptoms attributed in part at least to infarction of these organs. In sections of tissue from the brain liver spleen and bone marrow from such cases (prepared in the usual way by formalin fixation and haematoxylin and eosin staining) the parasites are usually revealed by clusters of fine brownish black pigment grains within the red cells in the capillaries and smaller blood vessels. Such an appearance is diagnostic of malarial infection with *P. falciparum*. Unless special methods of staining and fixation are employed the parasites themselves cannot be seen in such tissues.

Splenic puncture in malaria is often dangerous as haemorrhage may follow. However in blood obtained by puncture of the spleen or bone



FIG. 10.—Tetan malarial parasite one red cell showing malarial stippling (Todd)



FIG. 11.—Aestivo autumnal malarial parasite and small ring forms and crescents (Todd)

marrow the older trophozoites and schizonts may be found. The infected red cells are often shrunken and brassy in color. The schizonts occupy one half to two thirds of the red cell and show usually about 16 irregularly scattered merozoites with 1 or 2 compact masses of dark brown pigment.

The crescent shaped gametocytes are characteristic of this species and can often be found in the blood after a week of fever. In some cases young gametocytes may be ovoid rather than crescentic and in fresh preparations they tend to become swollen and ovoid as flagellation occurs.

**Plasmodium ovale** (of Mild Tertian Fever) —In 1922 Stevens described a new malarial parasite in East Africa and named it *Plasmodium ovale* and in 1930 Yorke and Owen reported that the morphological features were preserved when the parasite was passed by direct blood inoculation from one person to another. James Nieve and Shute also recognized *P. ovale* as a new species and succeeded in transmitting the infection through *Anopheles maculipennis*. Fairley (1933) reported further cases from West Africa, and Manson Bahr from Uganda.

Wilson and Wilson (1935) in a malaria survey in the northern part of Tanganyika Territory discovered 27 cases of malaria which they diagnosed as *P. ovale* infections. All except one of the patients were natives of the region and none was clinically ill. They stated that unless the parasites are present in fairly large numbers the differentiation of *P. ovale* from *P. vivax* may be quite impossible. Muhlens has reported 7 cases in which *ovale* like parasites were found at the Tropical School in Hamburg, some of which came from West Africa and South America. Muhlens while accepting this organism as a distinct species awaits further confirmation.

Gibbons (1933) described with figures 4 types of pigment seen in oocysts in the stomachs of naturally infected *Anopheles* in Uganda. On the basis of these he recognizes *P. ovale* as the fourth species on account of its dark brown pigment, coco bacillary in shape from 15-30 grains. Craig (1933) also accepts *P. ovale* as valid and believes that it is the same species which he described in 1900 in the blood of a soldier returning from the Philippine Islands but did not name it.

Sinton, Hutton and Shute (1939) suggest that the distribution of this parasite is confined almost entirely to tropical Africa though Blair has found cases in Southern Rhodesia. Eskin has described an infection contracted in Eastern Russia in which the diagnosis was said to be confirmed by Wenyon. and Muhlens (1938) has reported a case from Persia as well as from the west coast of South America. In such cases however Sinton suggests that *P. ovale* may have been confused with either *P. vivax* or *P. malaria*. On the other hand Giovannola (1935), Hauer (1938) and Friedmann (1938) point out that after repeated passages *P. ovale* may show a tendency to assume characters of *P. vivax* or *P. malariae*. Meloney (1936) believes that several points regarding *P. ovale* require elucidation before the specificity of it can be conclusively accepted. Ziemann also does not regard *P. ovale* as a separate species and points out that there is no region where *P. ovale* is exclusively found.

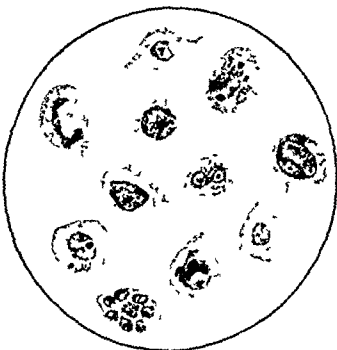
The clinical course of the disease in man according to Manson Bahr is a particularly mild one and the parasite tends to die out of its own accord. Fairley (1939) has reported a *P. ovale* infection in a fatal case of blackwater fever. It produces a tertian periodicity and Fairley points out that it differs from benign tertian in producing paroxysms of fever which come on in the evening or at night.

During 7 years Sinton, Hutton and Shute have induced primary infections with *P. ovale* in 108 patients in the Malarial Therapy Hospital in Epsom.

They found very little resistance among Caucasians to the acquisition of infection both by blood inoculations and by mosquito transmission. In a few instances they infected Caucasians who had previously been more resistant to infection with *P. vivax*, *P. falciparum* and *P. knowlesi*. They remarked that it is very curious to find in their one Negro patient that *P. ovale* usually considered to be the least pathogenic of the human species should give rise to active infection in this Negro when several other species of *Plasmodia* failed to do so. They found great infrequency of clinical relapses.

even in the absence of direct treatment in contradistinction to malar infection Mühlens (1938) however reports several relapses in *ovale* infections

**Morphology**—The young trophozoites of *P. ovale* are seen as small rings indistinguishable from the rings of other species They resemble those of *P. malariae* in showing but little amoeboid movement and they often tend to assume a band shape though band shapes are sometimes not observed The pigment is granular and blackish brown resembling that of *P. malariae* The medium sized forms are said by Stephens to be more characteristic The schizonts are smaller than a red cell containing from 6 to 12 merozoites usually 8 The gametocytes are oval and smaller than a red cell The infected red cells are only slightly swollen and are oval in shape They are so often oval that the shape is said to have special significance hence the specific name They have ragged fimbriated margins and even in the earlier stages of development of the parasites contain numerous Schüffner's dots Practically every infected corpuscle shows these dots and the corpuscles may become decolorized The sporozoites are said to be larger than those of *P. malariae*



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FIG 12—*Plasmodium ovale* Various stages of the parasite as seen in blood films illustrating the tendency to an oval shape the fimbriated margins and the platings of the red cells (After Dr Buchanan from case of Doctors D Meillon and Gear courtesy of Roy Soc Trop Med. & Hyg London)

**Plasmodium knowlesi in Macacus rhesus Monkeys**—This parasite which was discovered in the blood of a monkey by Napier and Campbell (1932) was named *P. knowlesi* by Sinton and Mulligan (1932) Brug (1934) emphasizes 2 morphological features not given in the original description (1) that the nuclei of the young parasites are usually surrounded by an unstained halo and (2) many band forms are found in

certain parts in thin smear preparations. These forms are often very irregular in distribution—usually in streaks in the thinnest portion of the film.

In the multiplication of the parasites the schizonts contain 6 to 10 merozoites usually 8 or 9. Basophile stippling of the red blood cells is common but Joly (1937) has found that the stippling is very variable as regards number and size. The numerous multiplication of the parasites in the peripheral blood within a few days indicated that a considerable portion of the multiplication must have taken place in the internal organs. Brug found that in some instances in 4 days as many as four of the five million red blood cells per cubic millimeter were destroyed and since this was a much greater loss than the number of parasitized red cells he suggested that red cell destruction was partially due to the toxin produced by the parasites.

This parasite causes almost invariably fatal infection in *Macacus* (*Silenus*) *rhesus* unless treated.

In man Milam and Kusch have found from inoculation experiments that the clinical course of the disease very closely resembles that of *P. vivax* malaria except that the term of infection is shorter.

Eaton (1938) has found that a specific agglutination of *P. knowlesi* occurs in the blood and is detectable both by macroscopic and by microscopic methods.

Agglutinins appeared in the serum of monkeys between 15 and 45 days after the onset of the infection and became progressively stronger as the malarial infection gradually subsided. The serum from normal monkeys and from monkeys chronically infected with a different species of malarial parasite such as *P. mui* did not agglutinate *P. knowlesi*.

As this species produces on inoculation into man a relatively mild and easily controlled infection it has been used extensively in the treatment of general paralysis.

## METHOD OF TRANSMISSION

Malaria is transmitted naturally by the bites of certain species of mosquitoes of the genus *Anopheles*. Hence the classification and identification of these species is important in the study of the disease.

**Mosquitoes**—All true mosquitoes belong to the subfamily Culicinae of the family CULICIDAE, a group of Diptera (or two winged insects) with many segmented thread like antennae (Nematocera) characterized by the peculiar venation of the wing (shown in Figs 14 and 15). The Culicinae include the only blood sucking Culicidae and differ from the other members of the family in having a long proboscis and scales on the veins of the wings and on most of the body and legs.

As in other insects the body consists of 3 main parts—the head, the thorax and the abdomen.

**The Head**—The space on the head behind the 2 compound eyes is described as consisting of two parts—that in front being called the frons and that behind the occiput. The nape is back of the occiput. The bulbous prolongation of the frons which projects over the attachment of the proboscis is the clypeus. The proboscis is straight in all mosquitoes of importance medically. In the male the puncturing parts are not

sufficiently resistant to penetrate the skin male mosquitoes feeding not on blood but on fruits and flowers instead. The proboscis consists of a fleshy scaled gutter shaped portion beneath known as the labium which terminates in two hinge joint processes—the labella. At the end of the labium is a thin membrane (Dutton's membrane). It is through this that filarial embryos are supposed to pass on their way from the interior of the labium to enter the person bitten. The labium may be considered

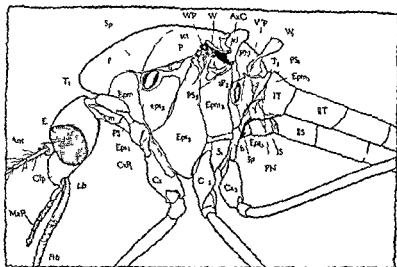


FIG 13.—Thorax of *Psephenus*. The individual segment of the thorax to which any part belongs is indicated by the small figure placed behind and below its symbol. The abdominal segments are distinguished by Roman numerals placed before the symbols of the parts. Arabic numerals placed before symbols signify number of order of repetition.

Ant antenna Ax axillary cord of wing base Cx coxa CxP pleural coxal process E compound eye Epw epimeral wing E s epimeral epimeral part of episternum of mesothorax Lh labrum (maxillary sclerite) Maxillary palpus P paraplegon PN notum (postscutellum metanotum) Prb proboscis PS pleural suture p c prescutum S sternum sc scutellum s s utum Sp spiracle T tergum W wing Ws haltere HP pleural wing process a small plate of mesopleuron b ring articulation of coxa b accessory plate of mesopleuron c lower part of metapleuron (After Howard, Dyar and Knab by courtesy of Carnegie Institution)

as the sheath of a knife holding and protecting the slender blade like penetrating organs. Lying in this groove we have from above downward the horse-shoe shaped labrum-epipharynx the under surface of which is open. This when closed by the underlying hypopharynx forms a tube through which the blood is sucked up by the mosquito. In the hypopharynx which somewhat resembles a hypodermic needle is a channel the venous salivary duct. It is down this channel that the malarial sporozoite passes. The proboscis is provided with a pair of maxillae and a pair of mandibles. These 4 structures with the hypopharynx and the labrum-epipharynx (6 structures in all) constitute the piercing parts. There are 2 pairs of mandibles and 2 pairs of maxillae on either side of the hypopharynx—the mandibles above and the maxillae below. The serrations of the maxillae are coarser than those of the mandibles. The sensory organs the palpi lie on either side of and slightly above the proboscis. These are of importance in differentiating mosquitoes and must not be confused with the antennae which are attached above the palpi and at the sides of the clypeus. These antennae also are of importance in distinguishing the sex of the mosquito. In the male the antennae are plumose in the female sparsely decorated with short hairs.

*The Thorax*—The thorax is largely made up of the mesothorax at the posterior margin of which is a small sharply defined piece the scutellum this may be smooth or trilobed. Underneath and posterior to the scutellum is the metanotum. This term implies that the structure belongs to the metathorax whereas in reality it is meso-

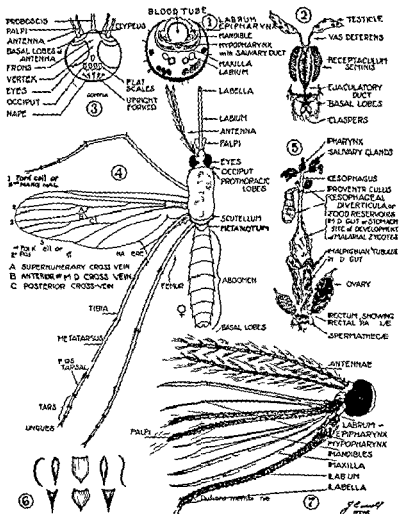


FIG 14 - A t my f th m gut do d k us a ou type of !

thoracic it is often called the postnotum or the postscutellum the latter term being usually applied in the higher orders. The metanotum is bare in the tribes Culicini and Anopheleini and has a tuft of setae in the tribe Sabethini. This holds true for the species found in the United States but not for the Neotropical species.

There is a pair of wings attached to the posterior part of the mesothorax and more posteriorly still a pair of halteres (rudimentary wings) attached to the metathorax.

The wing venation is important. The costa shows as a stout rib or vein bordering the upper side of the wing and running around the apex and lower border.

Below it has a fringe which may show spots. The location of the spots in the upper part of the costa of anophelines is of great value in differentiating species. Beneath the upper costal border the auxiliary or the subcostal vein runs to join the costa at some distance within the apex. The apex is the free end of the wing and the base that end attached to the thorax. Running parallel to the subcosta but reaching the apex is the 1st longitudinal vein. Below that is the 2d longitudinal vein which forks to make the 1st fork cell also called 2d marginal cell. (See Figs 14 and 15.) The third longitudinal vein originates from the 2d beyond the middle of the wing and is angulate at its base. The small transverse portion has been frequently called the supernumerary cross vein. The 4th longitudinal divides to form the 2d fork cell (2d posterior cell). The 5th and 6th longitudinal veins arise from the base of the wing and run to the periphery. A small cross vein which joins the basal part of the 3d vein with the 4th vein is called the anterior or mid-cross vein. Another cross vein joins the fourth vein and the upper branch of the 5th vein and is called the posterior cross vein. The posterior or

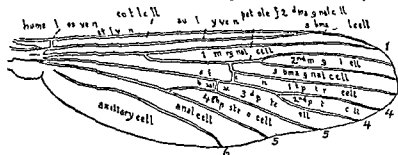


FIG. 15.—Venation of wing of *Culex*. (From H. Ward Dyar and Knab by courtesy of Carnegie Institution.)

basal cross vein is usually a short distance behind the anterior cross vein. It may however be in line with it or even beyond it. The petiole or stalk of the 2d marginal cell is of importance in differentiating genera. The wings of all mosquitoes have scales on the veins. In addition except in the genus *Uranotaenia* they also possess minute hairs microtrichia.

The 3 pairs of legs are attached to the thorax. Each leg has 9 parts of which the two short ones are the basally placed coxa and the small trochanter attached to it. Then comes the long femur, tibia and metatarsus with the 4 segments of the tarsus terminally. The last tarsal segment ends in 2 claws which in the female may be simple or unserrated.

**The Abdomen**—There are 10 segments in the abdomen. The genitalia arise from the 2 terminal segments as bilobed processes. The posterior abdominal appendages of the female are called the cerci; those of the male the hypopygium or terminalia. In the male there is a pair of lobe-like side pieces to which long curved appendages called claspers are attached. Lying between the side pieces are the sclerotized mesosome, the paired claspettes and the single anal lobe (in *Anopheles*) and the paired tenth sternites (in the other genera). See p. 1524.

**Development of the Mosquito**—In its development the mosquito undergoes a complete metamorphosis. The ova which are deposited in water or moist areas after several days give rise to the voracious rapidly growing larva which after 4 moults becomes transformed into the pupa or nymph which constitutes a non-growing stage in which the head and thorax are combined in an oval body. The duration of the pupa stage is often short usually only 1 to 3 days though some mosquitoes

have a pupal stage of several weeks. At the end of this time the pupa straightens out the integument splits dorsally and the insect emerges. After drying its wings for a time on its raft like pupal skin it flies away. The metamorphosis of the *Anopheles* mosquito in favorable weather

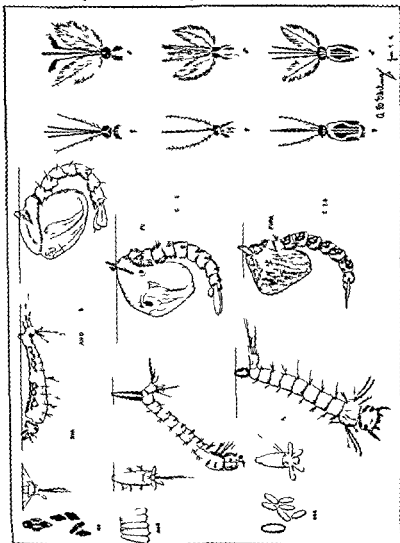


FIG. 10.—Life cycle of the *Anopheles* Cul. x and Ad.

conditions takes from 2 to 3 weeks: 1 to 3 days for the egg stage, 10 to 14 days for the larval stage, and 2 to 3 days for the pupal stage.

**Classification and Identification of Species**—Very definite information as to the identity of mosquitoes can be obtained if mosquito ova are



available for study by examining specimens in the several stages of development from ovum to imago. All points concerned in species differentiation are thus made available. Having determined the species from the characteristics of egg, larva and pupa, examination of the imago becomes a process of verification.

*Classification of Mosquitoes*—The classification of mosquitoes is steadily undergoing changes following progress in the science of entomology and the discovery of new species. Theobald's classification that has been the accepted one for many years is now found wanting and even misleading. The modern development in the study of larvae and their characteristics given by Dyar has helped much to bring order out of the

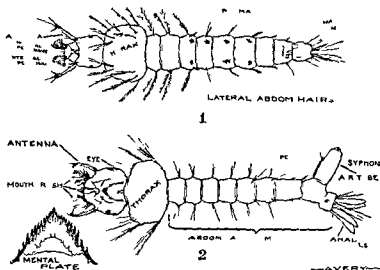


FIG 17—Asiphonate (*Anophelina*) larva *Anopheles* 2 Siphonate (*Culex*) larva *Aedes*

chaos that existed. The scope of this book does not permit us to include the large keys necessary to identify the mosquito species of the world. Some that may be mentioned which deal with the New World forms are found in the following references: King and Bradley 1939, 1941; Simmons and Aitken 1942; Komp 1941, 1942.

According to the latest general review of the mosquitoes of the world by Edwards (*Genera Insectorum* 1932), the 1400 known species of Culicinae are arranged in the 3 tribes Anophelini, Megarthini, and Culicini. The only tribes of medical importance are the Anophelini (Genus *Anopheles* including the species transmitting malaria) and the Culicini (genera *Culex*, *Aedes*, *Mansonia* and others, species of which transmit yellow fever, dengue, filariasis and some other diseases).

While keys are necessary for the definite identification of most mosquitoes, the worker in the field may find the following discussion useful in deciding whether a mosquito is a possible malaria transmitter or not.

*Anophelini*—With few exceptions the members of this tribe belong to the genus *Anopheles*. The adults have the scutellum evenly rounded behind (not trilobed as in

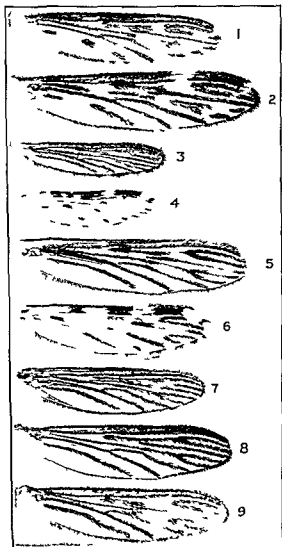


FIG. 18.—Wing of *Anopheles* (1) *A. ca* (2) *A. p. c.* (3) *A. b. br.* (4) *A. l. b. m.* (5) *A. m. a. ul. p.* (6) *A. p. d. p. t. pen.* (7) *A. t. pos.* (8) *A. w. alker.* (9) *A. g. d. m. l. lux.* (After H. W. Dyar and Knab by courtesy of C. M. G. I. St. Louis.)

the Culicini). The palpi of both sexes are about as long as the straight proboscis. At least the ventral side of the abdomen is without scales but in certain species these are also absent on part or most of the dorsal side of the abdomen. In most species the

wings are spotted. The male is distinguished from the female by the antennae which are sparsely provided with short hairs in the female highly plumose or feather like in the male. The tip of the abdomen also ends in the male in a pair of claspers which are lacking in the female.

The larvae lack a siphon at the posterior end the spiracles being sessile. They are surface feeders and lie parallel to the surface of the water. As a rule the pupa of *Anopheles* rests with the long axis of the first 2 abdominal segments nearly parallel to the surface of the water while in *Culex* and *Aedes* it is usually more nearly vertical. The eggs are provided with floats. The body of most *Anopheles* when resting on a wall usually forms a straight line at an angle of about 45°. Many *Anopheles* species do not stand in this position e.g. *atopos culicifacies*. Most species are twilight feeders. Many species hibernate as adults and there is considerable evidence that *P. miaz* may survive the winter (at least in milder temperate climates) in hibernating *A. maculipennis*.

**The Ova**—The *Anophelini* ova are oval in shape with pleated air cell projections laterally. They are laid upon the surface of the water to the number of about 100 which often form star shaped patterns. The egg stage is 2-4 days but shorter however in the tropics. The ova of *Culicini* mosquitoes are usually deposited in a scooped out raft like mass of about 250 eggs set vertically easily seen with the eye as in the case of *Culex* or laid singly on the ground or sides of containers as with *fedex*.

**Larvae**—There are two great classes of larvae—the siphonate and the asiphonate. The latter are always *Anopheles*.

The *Anopheles* larvae have a small head which is capable of being twisted around with lightning like rapidity. They are darker in color and have no siphon float parallel to the surface of the water have long lateral branching hairs and on the dorsum of the abdominal segments is a pair of palmate hairs which assist in keeping the larvae in the surface film. The larvae are usually called wrigglers. The duration of the larval stage is from 1 to 2 weeks according to the temperature.

*Culicine* larvae do not float parallel to the surface of the water but hang suspended at an angle with only the tip of the respiratory siphon pushed forward to the surface



FIG 19—Resting posture of mosquitoes 1 and 2 *Anopheles* 3 *Culex pipiens* (After Sambon) From P H Reports

from the axis of the body. The end of the siphon terminates in 5 pointed flaps. If you divide the length of the siphon by the breadth you get what is known as the siphon index. The larva of *Culex quinquefasciatus* has a long and slender siphon the larva of *Aedes aegypti* has a short and barrel shaped one. When at the surface the *Culex quinquefasciatus* larva has its siphon almost vertical and the body at an angle of about 45°. The *Aedes aegypti* larva hangs more vertically. As a rule the hairs proceeding from the sides of *Culex* larvae are straight and the head relatively large. There are also no palmate hairs along the sides.

**Pupae**—The pupa of the mosquito is an obdected one there being only a closely applied chitinous coating covering it it does not have a puparium as does the coarctate pupa of the house fly. The mosquito pupa is lighter than water while the larva is heavier. The *Anophelinae* pupa is distinguished from others by its widely flaring pupal trumpets. The trumpets of other species are usually slender and do not flare at the tip.

In the pupal stage it is rather difficult to differentiate species of mosquitoes from each other so that it is only of importance to recognize that the bloated appearing cephalothorax and shrimp like abdominal tail is a mosquito pupa. The most practical method for the identification of *Anophelini* species is to collect the larvae and later to study the adults which develop from the pupae.

The following species are important for the epidemiologist and are commonly encountered in the areas referred to

*Anopheles albimanus*—A medium sized grayish *Anopheles* the tip of hind tarsi white with a black spot on last joint. Legs blackish the fore tarsi with white rings at apices of the first three joints hind legs with apex of second and the third to fifth joints white a black mark on the fifth joint. About one half of second hind tarsal segment black remainder white. Wings with black and yellowish scales 2 large yellowish spots outwardly on costa other veins with small dark spots alternating with pale scales. Palpi long dark last joint and base of penultimate one white. This is the principal vector of malaria in tropical America.

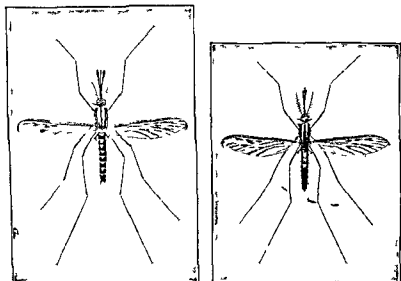


FIG. 20—Male and female *Anopheles maculipes* (After Castellani and Chittenden)  
(From P. H. Reppert)

*Anopheles punctipennis*—Legs black the tips of femora and tibiae with small pale rings and with small white spots. Wings with scales black except in certain spots as follows: A large one at outer third of costa and a smaller one at apex both involving second vein one on third vein in the cell on the stem and middle of both forks sixth vein white with black spot at base and tip. Widely distributed throughout North America it prefers to bite large mammals rather than man and is regarded as a relatively unimportant malarial vector.

*Anopheles quadrimaculatus*—A medium sized blackish *Anopheles* with black spotted wings. Tips of femora and tibiae whitish. Wings with the scales black forming 4 dark spots by being thickly placed as follows: Base of second vein in the cell on the cross vein and forks of second and fourth veins. Apex of wing uniformly dark. The principal vector in the United States. It occurs in the eastern states and the Mississippi Valley from the Gulf northward to New Hampshire and Wisconsin.

*Anopheles maculipennis* var. *scutellaris*—Wing much as in *A. quadrimaculatus* and without light spot at tip. A medium sized blackish *Anopheles*. Tips of femora and tibiae whitish. It is widely distributed in Europe northern Africa western and central Asia Alaska Canada and western United States. It is important as a transmitter wherever malaria occurs within its range. The variety *occidentalis* has a light spot in the fringe at the tip of the wing it is not believed to be a vector.

*Anopheles crucians*—A medium sized blackish *Anopheles* with mottled wings. Legs black with pale knee spots. Wings with a small yellowish white spot at apex and fringe.

wings are spotted. The male is distinguished from the female by the antennae which are sparsely provided with short hairs in the female highly plumose or feather like in the male. The tip of the abdomen also ends in the male in a pair of claspers which are lacking in the female.

The larvae lack a siphon at the posterior end the spiracles being sessile. They are surface feeders and lie parallel to the surface of the water. As a rule the pupa of *Anopheles* rests with the long axis of the first 2 abdominal segments nearly parallel to the surface of the water while in *Culex* and *Aedes* it is usually more nearly vertical. The eggs are provided with floats. The body of most *Anopheles* when resting on a wall usually forms a straight line at an angle of about 45°. Many *Anopheles* species do not stand in this position e.g. *atropis culicifacies*. Most species are twilight feeders. Many species hibernate as adults and there is considerable evidence that *P. vivax* may survive the winter (at least in milder temperate climates) in hibernating *A. maculipennis*.

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FIG. 19.—Resting posture of mosquitoes. 1 and 2 *Anopheles*. 3 *Culex pipiens*. (After Sambon.) From P. H. Reports.

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The following species are important for the epidemiologist and are commonly encountered in the areas referred to.

following the prevailing winds mosquitoes of this species had travelled up the coast 115 miles

Two years of severe dry seasons seemed to check the invasion but with the recurrence of normal rainfall there were severe epidemics in which the infection was transmitted by this species in localities over 200 miles west and north of Natal. In the Jaguaribe Valley of the state of Ceara there were said to be over 50 000 cases of malaria in 1938

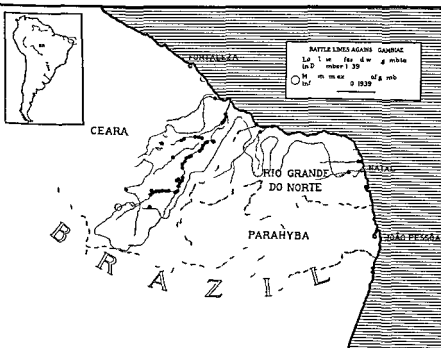


FIG. 21.—Distribution of *Anopheles gambiae* in Brazil (Courtesy of the Rockefeller Foundation)

According to the report of the Rockefeller Foundation over 90 per cent of the population was affected and mortality in certain districts amounted to 10 per cent. A subsequent report states that more than 5000 cases proved fatal.

An intensive campaign has been carried out by the Foundation in collaboration with the Brazilian Government for the eradication and prevention of further extension of the infection. The reports of Shannon and DeAndrade (1940) and Barber (1940) indicated the favorable results that had been accomplished by this campaign.

Barber found from gland dissections of the mosquitoes a sporozoite index of 2.7 to 10 per cent in different localities among the villages where *gambiae* were plentiful but malaria not yet present. On the other hand at Natal at the time of a severe epidemic a sporozoite index of 30.2 was found by Davis in the examination of 172 specimens.

other scales mostly black forming spots at the bases of the forked cells and three on the sixth vein separated by pale scales. The palpi of the female have the last joint whitish and a ring at base of penultimate joint. A relatively unimportant vector occurring in the South Atlantic and Gulf states and the Mississippi Valley.

*Anopheles pseudopunctipennis*—A large sized grayish *Anopheles* with white spotted wings. Legs black, knee spots yellowish white. Wings spotted in black and white, costa black with three white patches, third vein broadly white in the middle. Palpi of female with white rings at the bases of the joints. It greatly resembles *punctipennis* but is not really closely allied thereto. It is found in the southwestern United States, Central America and western South America to Argentina in which country and in Peru it is an important transmitter.

Another important transmitter of malaria of the New World is

*A. argyritarsis*—It is a South American species. Black costa with 2 distinct and several smaller white spots. Dark brown palpi with 2 narrow bands and a white tip. Legs with last 3 hind tarsal segments white; this species is not now regarded as a vector.

The following Old World species are also important transmitters of malaria.

*A. darlingi*—Wing similar to that of *A. albimanus*. Hind tarsi with last three segments all white. Palpi dark with 3 white rings, tip white. The most important vector of malaria within its range in South America. Recently discovered in Central America in Gulf of Honduras region. Easily confused with *A. albitarsis* and *A. argyritarsis*.

*A. funestus*—Wings with 4 yellow spots on a black costa and 2 black line spots on third longitudinal vein. Palpi with 3 white rings. Proboscis unbanded. Legs with faint apical bands. Common in tropical Africa but not in India.

*A. gambiae* (*A. costalis*)—Costa black with 5 or 6 small yellow spots. Palpi with 2 narrow white bands and white tip. Femora and tibiae with yellowish spots. Apical tarsal bands. Common in tropical Africa and Arabia; it has been introduced recently into Brazil. Both species are dangerous transmitters of malaria (and potential vectors of filaria). In one district Ross found 24 per cent of *A. gambiae* infected.

*A. stephensi*—Costa with 4 broad black spots separated by narrower yellowish spots. The corresponding second spot on the second long vein is narrower and divided into 2 unequal parts by a light spot (the smaller distal). Yellowish white scales over the dorsal surface of the abdomen, thorax and head. Legs black, the femora and tibiae spotted with white. White bands at intertarsal joints. Last hind tarsal segment black. It is abundant in India and a dangerous transmitter.

*A. maculatus*—Somewhat similar to the preceding. The abdomen is dark brown with golden brown hairs over the dorsum, and yellowish white scales over dorsum of thorax and head. The second spot on first long vein is divided into 3 parts by 2 pale spots. The white bands between the tarsal segments are broader and the last hind tarsal segment is white. Common and a dangerous transmitter in India, southeastern Asia and the East Indies but only in Shillong, India.

*Anopheles gambiae*—Special mention should be made with reference to the invasion of South America by the species *Anopheles gambiae*. The Rockefeller Foundation, 1939, has emphasized that it regards this mosquito as a most dangerous one. Although the species has been reported from Algeria and Morocco and from southern Arabia, its principal home is the African tropical belt extending from the southern border of the Sahara desert south to the Zambesi River, and it is said to be a scourge in central Africa. Until 1930 this species was not known in the western hemisphere. In that year or shortly before it crossed the ocean probably from Dakar either by airplane or on one of the fast French destroyers which at that time were working in connection with the French air lines between Dakar in West Africa and Natal in Brazil.

The species was first discovered by Shannon, 1930, at the port of arrival of these craft, in Natal in Rio Grande do Norte during a routine mosquito survey. In 1930-31 there occurred in the vicinity of the breeding area of it at Natal a very severe outbreak of malaria. By 1931

tions does not prove that the species is of practical importance as a transmitter under natural conditions

Thus *Anopheles atropos* a species which breeds in brackish water and is distributed along the Gulf and South Atlantic coast of the United States has been shown to be a good host from an experimental standpoint, but it has not yet been known to carry and transmit the plasmodia in the natural state

*A. punctipennis* and *A. crucians* abundant within their range prefer animal blood (are zoophilous) and are of minor importance as transmitters of malaria compared with *A. quadrimaculatus* which bites man and animals indifferently

As showing the uncertainty attaching to the question of a certain anopheline species being efficient hosts for malaria may be cited the case of *A. punctipennis*. This species has been frequently reported as incapable of transmitting malaria and Mitzmain reported experiments on 249 females of the species which had fed on crescent-containing blood and which were dissected from 3 to 38 days after such feedings with negative findings in stomach and salivary glands. Furthermore these mosquitoes failed to transmit malaria to healthy persons. Control experiments with *A. quadrimaculatus* and *A. crucians* were successful. In June 1916 King reported 33 per cent of positive findings after dissection of *A. punctipennis* which had fed on malignant tertian cases and 85 per cent of success where the man bitten had benign tertian malaria. These results showed as high a degree of success as that obtained with the control *A. crucians* and *A. quadrimaculatus*.

From the above it must be evident that there are other factors involved besides that of the host species. For example another factor which may influence the transmitting power is the feeding habits of the anopheline. One which is more voracious and fills and then ejects by rectum the blood taken from the malarial patient is more apt to be a transmitter than a species less voracious.

In certain instances there is a definite relation between the geographical distribution of different varieties or biological races of the species *A. maculipennis* and the occurrence of malaria. Swellengrebel and Hackett and Missiroli (1935) in contrasting the nonmalarial regions of northern Europe with the heavily malarious ones of southern Europe have demonstrated that although *A. maculipennis* is the only prevalent anopheline throughout this territory there are 5 or 6 recognizable varieties of it which may be identified by differences in their ova of which 2 *labranchiae* and *elutus* are always dangerous vectors of malaria even under extremely unfavorable conditions while the other varieties are associated with malaria transmission only under exceptional circumstances.

The different races of this species of mosquito may vary in their habits. In certain localities the mosquito is zoophilous and malaria is uncommon while in other (sometimes neighboring) regions androphilous strains occur and malaria is abundant especially if the insects pass the winter without hibernating instead of hibernating. As a rule the androphilous races have eggs with barred markings on the upper surfaces while the zoophilous races have uniformly colored eggs. In regions in which there are few domestic animals however the zoophilous races are driven to bite man and become important vectors as in certain regions in the Danube valley and in central Russia. As living conditions improve in such regions and animal husbandry is more extensively practiced these races revert to their natural hosts and malaria largely disappears.



Barber points out that the invasion of certain states of Brazil by *A. gambiae* has been followed sometimes after a lapse of several weeks by epidemic malaria, a result which might be expected where a very efficient vector entered a non immune population.

The Rockefeller Foundation has since (1941) reported the total extermination of this species from Brazil and this feat is said to rank among the greatest sanitary triumphs of all time. The complete story is told in the monograph entitled *Anopheles (gambiae) in Brazil 1930-1940* by Fred L. Soper & D. Bruce Wilson 1943.

**Efficient Mosquito Hosts**—In planning anti malarial measures it is important for an epidemiologist to recognize the different species of *Anopheles* that are efficient hosts in the community concerned. Of the various described species of *Anopheles* (some 180) according to Faust and Craig (1940) only about 60 have been incriminated of transmitting human malaria either by natural and experimental infection or on epidemiological evidence. Of these relatively few are of great practical importance. Hackett and Russell (1938) state that of the 180 odd species that may be considered as potential vectors (since all that have been tested become laboratory hosts of the malaria parasite) the great majority are rendered comparatively harmless in nature because their biting habits do not bring them into frequent and especially repeated contact with man. While in the United States and Europe only 2 or 3 species are of primary importance in India Paul Russell (1939) reports that 13 species are still under suspicion. Different races of *A. maculipennis* are the most important in Europe, as *A. quadrimaculatus* in the United States. The most important are listed on page 43.

Among the factors that may be of importance in the determination of efficient mosquito hosts are the breeding places of the insects and their distance from human habitations, the usual length of their flight, their abundance, seasonal prevalence, susceptibility to infection, their habits, choice of hosts (human or other animals), the percentage of human blood in the stomach of the insects, their occurrence and tendency to enter human habitations and other houses, the time at which they bite and the question of a high sporozoite index and whether the sporozoites are degenerated.

Barber, who has had an unusually great experience (1937) in mosquito campaigns and malarial epidemiology, points out that an investigation of many of these criteria is sometimes necessary to determine an efficient host, though it may not be necessary always to study them all in detail. Thus a comparatively short study of *A. gambiae* (*costalis*) in West Africa was sufficient to indicate to Barber and Rice that this mosquito was an effective carrier there, especially on account of its attraction to human dwellings, the percentage of human blood in the stomach and the high sporozoite index. However in some localities a very long study is necessary to acquire satisfactory evidence.

The malaria indexes of the human population may give important evidence, especially where the occurrence of several mosquito species varies greatly in locality or season. The parasite index of very young children may be especially helpful in this respect, as it sometimes indicates more completely the season of malaria transmission.

The fact that the malarial parasite can develop in a given species of mosquito and that the latter can infect man under experimental condi-

species responsible for the transmission of human malaria has been known for some years every few years it has been necessary either to add new species of effective transmitters to the list or to extend the geographical area in which a species has been found of importance For example Simmons (1936) has added *A punctimacula* as an important transmitter of malaria in the Canal Zone Panama and Shannon (1932), in connection with a severe outbreak of malaria in the City of Natal Brazil discovered the presence of *A gambiae* (*costalis*) Also Roebboom Fox and Laird (1941) report *Anopheles bellator* naturally infected in Trinidad

### MALARIA CARRYING MOSQUITOES Species Known to Carry the Malarial Parasite

In the following table the classification of Lt Colonel W H W Komp for the Western Hemisphere and of Lt Colonel Paul F Russell for Africa Asia Japan and the Far East has been followed

NORTH AMERICA	NORTH AFRICA NEAR EAST AND RED
<i>A crucians</i> (unimportant)	SEA AREA
<i>A maculipes</i> var <i>freeborni</i> (important)	<i>A claviger</i> ( <i>bifurcatus</i> )
<i>A punctipennis</i> (unimportant)	1 <i>labanchaei</i> <i>branchiae</i>
4 <i>A. adrimaculatus</i> (most important)	<i>A multicolor</i>
SOUTH AND CENTRAL AMERICA	4 <i>phaeosis</i>
<i>A albimanus</i> (important throughout Caribbean region)	<i>A sahaiensis</i> ( <i>elutus</i> )
<i>A albistis</i> (locally important)	<i>A sergenti</i>
<i>A bellator</i> (Trinidad)	<i>A superpictus</i>
<i>A darlingsi</i> (Gulf of Honduras region South America)	CENTRAL AND SOUTH AFRICA
<i>A gambae</i> (now reported to have been exterminated from South America)	4 <i>f. nestus</i>
<i>A punctimacula</i> (Syn <i>A. malefactor</i> )	<i>A gambiae</i>
Central America not important	<i>A gambiae melas</i>
<i>A pseudopunctipennis</i> Mexico and Central America and West Coast of South America N Argentina Locally important	<i>A hancocks</i>
<i>A. taeniaculatus</i> (included under this name in the literature are six or more species <i>A. asolensis</i> <i>emiliani</i> <i>oswaldo</i> etc. of wide distribution in South America the exact relation of which to malaria is yet undetermined)	4 <i>hargreavesi</i>
EUROPE	4 <i>moucheti</i>
<i>A. algeriensis</i>	4 <i>moucheti nigerensis</i>
1 <i>bifurcatus</i>	4 <i>nili</i>
4 <i>hyrcanus</i>	<i>A phorcensis</i>
<i>A. hyrcanus</i> var <i>pseudopictus</i>	<i>A. pretoriensis</i>
<i>A. maculipennis</i> vars <i>aloparus</i> <i>labanchaei</i> etc.	PERSIAN GULF AND CAUCASIAN AREA
<i>A. plumbeus</i>	<i>A. sackroni</i> ( <i>elutus</i> )
<i>A. merseae</i>	<i>A. stephensi</i> (type?)
<i>A. sackroni</i> Favr ( <i>elutus</i> )	1 <i>superpictus</i>
<i>A. superpictus</i>	AFGHANISTAN BALUCHISTAN INDIA AND
	CEYLON
	<i>A. annulipes</i> ( <i>fuliginosus</i> )
	<i>A. culicifacies</i>
	<i>A. ficalis</i> ( <i>littoralis</i> )
	<i>A. jeyapensis</i>
	<i>A. leucosphyrus leucosphyrus</i>
	<i>A. mnisus</i>
	<i>A. philippinensis</i>
	<i>A. stephensi</i>
	<i>A. sundanicus</i>
	<i>A. superpictus</i>
	<i>A. taeniaculatus</i>

The type of breeding place of the particular species is also of fundamental importance. Thus *A. quadrimaculatus* in the United States breeds in quiet pools or swamps. Thorough drainage of such areas largely eliminates this mosquito and with it malaria. The reverse is true of some other species in other localities. Thus *A. maculatus* breeds in open flowing streams and in certain districts in Malaya it has been possible by damming up the streams and producing pools to eliminate this vector and replace it with harmless species. In Europe some of the races of *A. maculipennis* which transmit malaria breed indifferently in flowing or stagnant water and drainage projects which are successful in America have proved quite ineffective. The breeding places of the same species may differ in different localities. Thus a race of *A. ludlowi* (*sundensis*) which according to DeLangen and Lichtenstein is the most dangerous anopheline mosquito in Java appears to prefer to breed in salt water, and this race is largely limited to the coast but in Sumatra a race which breeds in fresh water is an important transmitter throughout much of the island.

Although it has been shown that some *Anopheles* mosquitoes are capable of travelling considerable distances (even 20 miles has been reported and  $1\frac{1}{2}$  miles has been demonstrated) as a rule their range of flight is small (not over a mile) and they are rarely found in large numbers unless there are suitable breeding places in the immediate vicinity. A flight distance of 100 yards between the breeding place and the home is common.

Khglar in Palestine classified the flight of *Anopheles* in 3 grades: direct flight about  $1\frac{1}{2}$  miles; about 2.5 kilometers; range of dispersion during the breeding season 3.5 kilometers; hibernating flight up to 8 kilometers. In general it has been thought that some 1000 or 1500 yards of water between a ship and a malarious coast is sufficient to secure immunity to those on the ship. Mauson Baht has pointed out that the intervention of a belt of trees between a malarious swamp and a village sometimes gives much protection by filtering out the mosquitoes. High humidity also favors human infection because in a period of hot dry weather most of the adult mosquitoes perish before they become infective.

For reasons which are not well understood the same species may be an important transmitter in one region (e.g. *A. subpictus* (rossi) in the East Indies) and practically harmless in another (India) even though it is abundant. Also in Argentina *A. pseudo-punctipennis* is extremely important but in Central and North America it has been reported as a relatively ineffective species. In the Federated Malay States *A. maculatus* is the most important carrier and *A. ludlowi* is only of minor concern but in Sumatra the conditions are reversed and in the Philippines *A. minimus* is much more important than *A. maculatus*. Even in the same locality a change in local conditions which favors the propagation of the mosquito may result in converting a species previously harmless into a dangerous transmitter. This occurred when the cultivation of rice was introduced into Sumatra after the war. An epidemic of malaria promptly broke out transmitted by *A. hyrcanus*. It had been known that this species was present but it had been considered harmless because it breeds abundantly in the rice fields of Java and other neighboring islands and there it is not regarded as a transmitter. Such points can be determined only by local epidemiological studies and not alone by laboratory experiments or by analogy from conditions in other regions.

In connection with the table of efficient mosquito hosts it should be emphasized that while the role played by the more widely distributed

reference to the infection of mosquitoes. They are not usually infective to the insect when they first appear in the blood but must be several days old before they become infective. However great variations have been found in the infectivity of different mosquitoes. James (1931) was unable to infect *A. maculipennis* when it was fed on patients with gametocytes as high as 12 to 100 leucocytes while other patients having only one gametocyte to 200 leucocytes were found infective for this species. The reason for this fact is not clear but it is suggested that the gametocytes may vary in their power to infect. Also it has been found by different observers that mosquitoes become more frequently infected from some patients than others.

Other factors concerned in epidemiology are race, age, sex, occupation, climatic environment, meteorological conditions and altitude. Thus races that have long been exposed for many generations to malarial infection have apparently acquired a greater resistance to infections than people who have never been subject to the disease. It is a well recognized fact in the tropics that such individuals appear to have a relatively greater immunity to malaria than the majority of white people yet many of them carry parasites in their circulation and serve as carriers. This question will be discussed under racial immunity p. 60.

The native children to a striking degree harbor parasites and to them in many tropical localities malaria is a prime cause of death. After repeated infections if they do not succumb a temporary immunity usually is acquired and they also may act as carriers.

*Age* is of some importance in epidemiology first because children usually show a higher malarial infection rate in a malarial community than adults hence they are of value as a means of estimating the amount of malaria in a community and of course those with the gametocytes in their blood constitute a factor in the further spread of the disease.

*Sex* is of interest only because there are usually more infections among men because men are often more exposed to the bites of infected mosquitoes in their occupations.

*Occupation* is of importance in that laborers in camps, about plantations, in various engineering constructive work, in agricultural pursuits and soldiers in the field in the tropics are brought into greater contact with infected mosquitoes.

There are many important epidemiological factors which relate to the development of the malarial plasmodia in the mosquito. Some of these have been discussed under efficient mosquito hosts. Only mosquitoes belonging to the genus *Anopheles* have been shown to transmit the human plasmodia and only certain species of this genus are natural transmitters. Hence it is necessary that at least one of these species must be present in the locality in order that malaria may be transmitted there.

Many localities in the tropics owe freedom from malaria to the absence of anophelines as for instance Tahiti. Sometimes malaria bearing mosquitoes may acquire the habit of feeding on animal blood other than that of man. Thus Barber and Rice (1937) have shown that there is in Cyprus a high malaria index in many villages where *A. superpictus* can

BURMA MALAYA THAILAND INDO CHINA	NETHERLANDS EAST INDIES
SOUTH CHINA AND FORMOSA	<i>A. aconitus</i>
<i>A. aconitus</i>	<i>A. barberostris</i> (var. <i>vanus</i> ? in Celebes)
<i>A. culicifacies</i>	<i>A. hyrcanus nigerrimus</i>
<i>A. hyrcanus sinensis</i>	<i>A. leucosphyrus leucosphyrus</i>
<i>A. jeyporiensis condidiensis</i>	<i>A. kochi</i>
<i>A. maculatus</i>	<i>A. maculatus</i>
<i>A. minimus</i>	<i>A. minimus</i>
<i>A. sundanicus</i>	<i>A. subpictus</i> (probably var. <i>malayensis</i> Hack.)
<i>A. umbrinus</i>	<i>A. sundanicus</i> (salt water ludlows)
JAPAN NORTH AND NORTHEAST CHINA	<i>A. umbrinus</i>
KOREA	AUSTRALIA NEW GUINEA PACIFIC ISLANDS
<i>A. hyrcanus sinensis</i>	<i>A. bancrofti</i>
<i>A. labranchiae atropartus</i>	<i>A. punctulatus punctulatus</i>
<i>A. polioni</i>	<i>A. punctulatus moluccensis</i>
<i>A. ascharovi Favr. (clutus)</i>	
THE PHILIPPINES	
<i>A. mangyanus</i>	
<i>A. minimus flavicostis</i>	

Note.—The above table does not include all species which have been experimentally infected or only occasionally found naturally infected or suspected on purely epidemiological grounds. It must also be noted that apparently the same species may be a vector in one area but not in another.

**Epidemiology**—The requirements for the spread of malaria are (1) Human beings who have sexual forms of the malarial parasite in their peripheral circulation (2) efficient anopheline hosts and (3) a sustained mean atmosphere temperature above 60 F (16 C). Whatever favors the presence and increase of efficient anopheline mosquito hosts and the access of these malaria infected insects to human beings favors the spread of malaria.

One condition necessary in order that man may be infective to the mosquito is the presence of gametocytes in the peripheral blood. Not all individuals infected with *Plasmodium* have gametocytes in their blood during certain periods. In a series studied by Craig gametocytes were found in a little over 33 per cent of those infected with *P. falciparum* and in about 50 per cent of those infected with *P. vivax*. Such individuals may be termed carriers of malaria. But in order for the individual to be a source of infection not only must the gametocytes be present but there must be a sufficient number of them to infect the mosquito. Darling found that the mosquito did not tend to become infected unless the carrier had more than 12 gametocytes to the cubic millimeter of blood while Green found that in order to infect *A. maculatus* the blood must contain at least one gametocyte of *P. falciparum* to 200 leucocytes one of *P. vivax* to 1000 leucocytes and one of *P. malariae* to 330 leucocytes. However the exact number necessary for infection varies under different conditions with different mosquitoes.

The proper proportion of microgametocytes and macrogametocytes is of importance. When macrogametocytes were greatly in excess of the microgametocytes there was a reduction in the number of mosquitoes that became infected. The age of the gametocytes is another factor with

Lt Kenneth L. Knight USNR in a personal communication (1944) reports that the sole anopheline found to date in the New Hebrides Islands is *Anopheles punctulatus farauti* (= *Anopheles punctulatus moluccensis* of the New Hebrides and Solomons) previously mistakenly identified there as *Anopheles punctulatus*. In the Solomo Islands *Anopheles punctulatus farauti* appears to be the sole vector even though other anophelines have recently been discovered there.

proves that temperatures approximating freezing ones will fail to destroy the parasite of hibernating mosquitoes

Mosquitoes may hibernate and possibly cause new infections the following spring. On account of Mitzmain's negative experiments with hibernating mosquitoes some observers believed that cases of malaria in the spring are usually due to relapses and that man is usually the winter carrier. However a number of other observers have shown that it is possible for the plasmodia to remain alive in the mosquito throughout the winter and renew their development in the spring.

Wenyon found experimentally that mosquitoes which had fed on malarial blood and were kept at incubator temperatures for a week to allow development of zygotes showed inhibition of development of zygotes when kept at temperatures corresponding to hibernating ones. This treatment did not kill the zygotes and complete development took place when subsequently the mosquitoes were again subjected to incubation temperatures. From this and other observations it would seem evident that the plasmodia may sometimes remain viable during the winter hibernation and may renew their development in the spring. However Mitzmain regarded hibernation as destructive to zygotes.

Climatic environment is important in regards both the frequency of malarial infection and its severity. It has been pointed out that in the warmer countries *P. falciparum* is usually the prevailing species where the temperature and humidity favor its development in mosquitoes and where moisture, temperature and rainfall are especially favorable for the breeding of the mosquito host. In such areas the disease is often endemic and common and pernicious infections are frequently observed. On the other hand in temperate climates where the temperature conditions do not favor to the same extent the development of *P. falciparum* in the mosquito the milder infections with *P. vivax* prevail. The climatic conditions in such regions being more favorable to the development of this species in the mosquito.

The effect of climatic conditions is also shown in the seasonal prevalence of malaria, the disease being absent usually in winter in the temperate zones but present throughout the year in the tropics. However in the tropics malaria is more prevalent usually during the beginning and toward the end of the wet season and less so during the latter part of the dry season.

In the southern United States Boyd and Kitchen found in experimental therapeutic inoculations of 155 white patients by mosquitoes infected with *P. vivax* that the largest proportion of successful inoculations were secured during the summer months of July, August and September. The incubation periods were the shortest and the clinical attacks longest and the liability of recrudescences greater. The largest proportion of unsuccessful inoculations occurred during January, February and March.

In Macedonia Barber and his associates (1936) have found that transmission of malaria begins definitely during the months of May and June and reaches a maximum in the period of July through September. Transmission during October and November was low, hardly above that of winter. The species of *Plasmodium* among infected infants showed a marked seasonal incidence, *vivax* predominating in spring and *falciparum* and *malariae* in late summer and autumn.

be the only vector. In that island, domestic animals are relatively few in villages during the summer. The deviation to animal hosts is only about one sixth of that which takes place in Macedonia and the sporozoite index is nearly 7 times greater. However in parts of Macedonia they find that *A. superpictus* plays a minor role in the transmission of malaria, since here in spite of its abundance and its relatively high sporozoite index the species is especially attracted to domestic animals as hosts in preference to man.

It is well recognized that rural populations are more liable to malaria than those of towns and as the population of a country moves to the industrial centers human blood may become difficult to obtain and the anophelines turn to other sources of blood supply. It has been suggested that mosquitoes may suffer from other infections which may be inimical to the development of malarial zygotes or that certain vegetable food of the female may destroy them. Sinton and Shute (1938) have shown that under favorable conditions of environment *A. maculipennis* infected with *P. vivax* show no higher mortality than uninfected mosquitoes. Death from infection with *Plasmodia* is therefore not responsible for the fact that certain species are not natural transmitters. The sporozoites in some species and at certain seasons become degenerated in the mosquito.

Anophelines bite chiefly in the twilight and at night and in connection with this fact the shutting of windows toward nightfall has been the custom for ages in many malarious parts of the world. During the day they select some dark place or dark colored wall for sleeping. Hence the advantage of a buff-colored wall interior.

Anophelines do not like wind and often seek the protection of underbrush. As regards distance of flight from breeding places which has already been referred to (usually not over a mile) Metz has noted that *A. crucians* were not distributed generally beyond 7000 feet. They were rarely encountered between 7000 and 9000 feet beyond which distance they were not found. Watson and Spain regard the maximum flight of *A. quadrimaculatus* in the southern United States as one mile.

While anophelines are usually rural or at any rate prefer the suburbs of cities one must differentiate between domesticated and wild anophelines these latter keeping away from man and consequently not playing a transmitting role.

Temperature is a most important epidemiological factor for unless temperatures are favorable, development of the plasmodia in the transmitting mosquitoes will not occur. The most favorable temperature for development of the mosquito varies according to the species of plasmodia.

The zygote of *P. malariae* develops at a lower temperature than *P. vivax* and *P. falciparum*. The optimum temperature for the development of *P. vivax* in the mosquito has been given as 25 C (77 F) and complete development will occur in about 15 days at this temperature. For *P. malariae* the optimum temperature has been given as 22 C (72 F) and development is complete usually in from 18 to 21 days. For *P. falciparum* the optimum temperature has been reported as 30 C (86 F) and development is complete in from 10 to 12 days. The malarial zygote will not develop in the stomach of the mosquito if the temperature is below 16 C (60 F). Our earlier views as to temperature requirements for the development of zygotes in the mosquito must be changed as King has shown that *P. vivax* sporonts will survive exposure to temperatures of 30 F for two days and *P. falciparum* temperatures of 35 F for one day. This

Small collections of water or sluggish clear streams having a border growth of grass or rushes are preferred by many species of *Anopheles* for depositing eggs

The pools made by excavations following railway or other similar construction are favorite breeding places (borrow pits). In many such locations the small fish or tadpoles which prey on the larvae cannot work their way through the obstacles and again petroleum oil cannot be easily distributed in a network of grass. Anophelines of different species and of different countries seem to vary much in their selection of water for depositing their eggs. On the whole culicines do not seem to object to foul collections of water while the anophelines generally avoid such breeding places. In Trinidad B.W.I. a malaria vector breeds in water holding bromeliads in the cacao plantations. However one should not generalize but go out and search for breeding places. Paul Russell points out that in India the vectors of this disease breed not only in classical marshes and ponds but in rain water in river pools in seepage in salt water in wells and cisterns and even in hoofprints.

### PATHOLOGY AND MORBID ANATOMY

**Blood**—In fatal cases of malaria the pathological lesions are those connected with the destruction of enormous numbers of red cells not only the infected red cells being destroyed but also others not so parasitized. It has been suggested that at the time of sporulation and rupture of the merocyte a toxin is set free and that haemolysins and endothelolysins are produced. Brown believes that the malarial pigment (melanin or haemozoin) acts as a haemolysin and by being taken up by endothelial cells brings about their degeneration with associated capillary haemorrhages. All three factors—red cell destruction by parasites haemolytic action on red cells and capillary haemorrhages lead to anaemia.

The anaemia which is dependent upon the severity and length of the attack of infection may be slight or severe. In mild acute infections it may not be apparent but since malaria is always associated with haemolysis some degree of anaemia is usually present. The most serious form occurs in malignant subtertian infections where the haemolysis may be so intense as to cause blackwater fever with severe haemoglobinuria. Manson Bahr points out that after a severe single paroxysm of malignant tertian malaria a blood count may show that as many as one million red cells per cubic mm. have been destroyed and this destruction may go on until the blood count may reach only a million or even less. However the anaemia is generally not prominent in mild benign tertian and quartan infections. In chronic malaria in Caucasians Fairley (1934) in some 30 cases of malignant tertian infection studied in England found that the anaemia was not severe the average number of red cells being 3 826 000 per cmm. and the average haemoglobin about 73 per cent.

In addition to the malarial oligocythaemia changes may occur in the red cells themselves as poikilocytosis anisocytosis stippling and polychromasia. In stained preparations by Romanowsky's method from chronic cases of malaria the presence of blue or purplish dots and granules (stippling punctate basophilia) is not uncommon. In the earlier literature on malaria this basophile punctation was regarded as a degenerative

Tadpoles are generally vegetarians and do not eat mosquito larvae. However there is an exception in the tadpole of the spadefoot toad (Kemp).



Soil moisture and altitude influence the prevalence of malaria, according to whether they are or are not, suitable for the breeding of efficient mosquito hosts. It is a well known fact that in many malarious countries so long as the soil remains undisturbed cases of the fever may be comparatively rare but when building construction of roads and other operations implying soil disturbance are undertaken, then severe attacks of malaria may occur. This is particularly because soil disturbance usually implies the formation of excavations and holes in which puddles of water occur favorable for the breeding of mosquitoes. For the same reason earth cutting in the clearing of jungles produces changes in the general physical appearance of the locality which may favor the introduction of especially efficient species of mosquitoes of transmission. Hence it is obvious why malaria is more common in flat low lying, marshy regions in the terrain along the foot of mountain ranges and along the deltas of large rivers when people reside in such localities rather than in well drained uplands and carefully cultivated districts which are more often malaria free.

Altitude *per se* has apparently little influence on malaria up to several thousand feet but the disease is usually rare at high altitudes. However in a number of tropical countries malaria has been reported as indigenous in high altitudes although not prevalent.

Thus it has been found in the Far East in the Himalayas at 3000 feet in Southern India at between 5000 and 6000 feet in Africa in the Belgian Congo in the plateau of Ituri at 5000 feet and at Ruwenzori at 6000 feet. The highest endemic altitude reported has been at 9000 feet in Quito Ecuador. Morin and his associates (1936) have found that malaria is very severe and blackwater fever quite prevalent in Indo China at an altitude of 5000 feet. They believe that malaria was introduced to this area by soldiers and immigrants from hyperendemic areas at lower altitudes. *A. sinensis* was found to be the vector. At altitudes of 3500 feet in many parts of the tropics malaria may be fairly frequent. Mirra (1937) has studied the disease at this altitude in Guatemala where *P. falciparum* was the parasite concerned and *A. pseudopunctipennis* was found to be also a transmitter. Lega (1938) in Italian East Africa at altitudes between 3000 and 3500 feet found malaria very prevalent with spleen rates among the population up to 90 per cent. Callomet (1936) found in the Ituri at 5000 feet that *A. gambiæ* and *A. christyi* were breeding in small ponds created by dams placed across ravines to impound water for agricultural pursuits. White (1937) has especially studied the epidemiology of malaria in the Jeypore Hill region of Bengal. Twelve species of *Anopheles* were examined but only 3 of the species were found to have natural gland infections. These were *A. minimus minimus* var. *varuna* and *A. flustrilis* (Histon). Malaria in this region at an altitude of 3000 feet was transmitted entirely by these species. Stratman Thomas (1938) in Cyprus found that spleen and parasite indices were highest at altitudes of 1000-1500 feet. Above 2000 feet the parasite rate was considerably higher than the spleen rate.

Altitude importantly diminishes the amount of malaria by lowering the temperature. Thus in Europe and the northern United States malaria does not occur in situations greatly above sea level. In addition altitude may also exert influence upon drainage since at higher levels accumulations of water suitable for the breeding places of mosquitoes are less extensive.

Colonel I. A. Fox points out that in Kenya the Malaria endemic areas generally extend to altitudes of 6000 feet and in sheltered mountain valleys *A. Gambiæ* breeds freely and causes severe outbreaks of malaria at elevations extending to 8000 feet above sea level. (Report to the Surgeon General Sanitary Survey 1942)

that a sustained submaximal reticulocytosis is very characteristic of an actively persisting malarial infection

Eaton (1934) noted that the reticulocytes were especially invaded by the parasites of benign tertian malaria and that they were more fre-

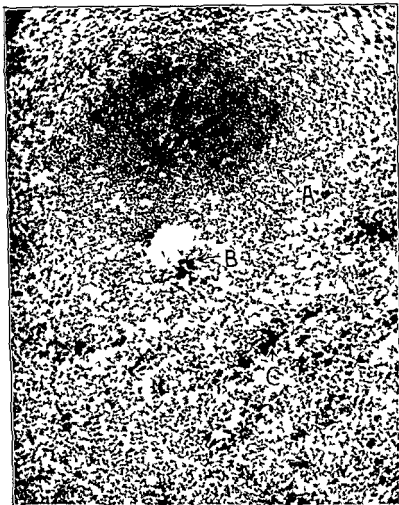


FIG. 2.—Section of splenic tissue showing (A) staining reaction of pigment in the Malpighian corpuscle (B) pigment in artery of Malpighian corpuscle and (C) marked mottling of splenic pulp.  $\times 25$  (Army Medical Museum phot. N. 46957)

quently infected than the mature red cells. It was suggested that the erythrocytes became infected only when in the reticulocytic stage. Jacobsphal confirmed this report and stated that in new infections with

change. However Whitby and Britton (1937) regard stippling as a sign of immaturity and due to the large amount of reticulum within the cell. Obviously this condition should not be confused with the basophilia in which the basophils or mast cells, are increased. These cells also formerly regarded as degenerated cells, have been shown by Sabin to be normal cells. Other alterations in the infected red corpuscles may also occur and will presently be considered.

The anaemia in chronic malarial infections is caused by the continuous slight haemolysis, and the bone marrow is constantly stimulated to replace the destroyed cells. It is common therefore for the blood to contain a considerable number of reticulocytes, even without treatment. This feature is sometimes so striking as to give rise to difficulties in diagnosis unless malaria is suspected since reticulocytosis with a hypochromic anemia is found also after haemorrhage in acholuric jaundice and in lead poisoning.

Upon examination of the peripheral blood in *P. vivax* infections corpuscles may be found containing at different times all the different stages of the parasite. As the parasite grows in size the red cell enlarges and becomes paler. Sometimes the corpuscles appear to be nearly twice the diameter of the healthy cells. If the preparation is heavily stained for example with Giemsa's solution there may be seen in the infected corpuscles a fine red stippling (Schüffner's dots). These are usually more plentiful, much smaller and more constant than are Maurer's dots observed in *P. falciparum* infection. However in some of the older *vivax* parasites Schüffner's dots may also become coarse and prominent. The presence of Schüffner's dots was formerly regarded as diagnostic of the species but they do not always occur in *vivax* infection and they have been noted in *P. ovale* infections.

In infections with *P. ovale* the red cell may be oval in shape with irregular margins. It is pale but seldom strikingly enlarged. In infections with *P. malariae* the red cell is not enlarged sometimes it appears slightly smaller than the surrounding cells. It does not show constant color changes. Schüffner's dots and Maurer's dots are not present but James has sometimes noted in lightly stained specimens indistinct dots or points which he terms Ziemann's stippling.

In *P. falciparum* infection the only forms of the parasite which are usually found in the peripheral blood are the ring forms or crescents or both. The red cells which contain ring forms of the parasite are not enlarged but the red cells which contain crescents are frequently only visible as a pale disc extending across the concavity of the crescent. The other corpuscles are sometimes copper colored and there is not the fairly constant pallor seen as in chronic *vivax* infection. Some of them contain coarse dots which usually stain violet in color and may vary considerably in size. Sometimes these appear as irregular mottling and give the appearance of clefts in the protoplasm. These (Maurer's dots) are regarded as diagnostic of *P. falciparum* infection. However they are frequently absent and are usually only found when the infection is heavy. In chronic infections true megalocytes are sometimes seen in the blood as well as very minute dark colored spherical corpuscles which may be nucleated. Also in the chronic cases there is a decrease in the blood volume and in addition to the destruction of the red cells there may be a marked haemoglobin diminution of the surviving corpuscles to 50, 20 or even 10 per cent (Haldane). As a result of this there may be a diminution of the total haemoglobin content to a greater degree.

Fairley (1934) has especially called attention to the reticulocytosis which may occur in malaria and has emphasized that a reticulocyte crisis may follow the proper control of malaria with quinine. He believes

*P. falciparum* parasitize the erythrocytes of all ages and that the number of adult erythrocytes parasitized are much greater than that of the reticulocytes. Kitchen believes that Wright's stain for vital staining of reticulum in immature erythrocytes by the dry slide method is as satisfactory as brilliant cresyl blue.

The leucocytes are usually increased during the actual malarial paroxysm but subsequently when the temperature has reached its highest point and toward the end of the attack they become diminished, sometimes to as low as 2000 per cmm. After the malarial attack the leucocyte count may again become normal. However the characteristic picture of chronic malaria is that of a moderate leukopenia with an absolute increase in the monocytes. In fact a leukopenia with a monocytosis of 15 or 20 per cent is suggestive of malarial infection. The greatest increase in monocytes takes place during the apyrexial periods.

**Phagocytosis**—Malarial pigment remnants of degenerating parasites and red blood corpuscles are often taken up by the polymorphonuclear leucocytes and monocytes. Much destruction of the red blood corpuscles both normal and those containing parasites takes place in the spleen. This process of blood destruction is believed to be one of the principal causes of the malarial anaemia.

The large number of pigmented macrophage cells seen in the splenic sinuses are a characteristic feature of the pathology especially of infection with *P. falciparum*. The presence of pigment containing leucocytes in the peripheral blood gives evidence that the patient is infected with malaria.

**The Viscera**—The most striking characteristic feature in cases which have died after protracted infection is the slaty or blackish pigmentation of the organs especially of the spleen, liver, brain and sometimes the intestinal mucosa. It is due not only to the parasites themselves containing grains of pigment but especially to the quantities of pigment set free from disintegrated parasites and present in innumerable phagocytic cells both of the type of wandering macrophages and of the endothelial cells of the capillaries.

The malarial pigment which is present in the body of the parasites is probably derived from the red cell. Since it only arises as the result of growth and metabolism the young ring forms do not show any pigment, the pigment increasing in proportion as the trophozoite grows at the expense of the red cell. The conditions known as Schüffner's and Maurer's dots result from an altered staining reaction on the part of the cytoplasm of the red cells themselves. They are not in the parasite and are not due to pigment.

This malarial pigment is insoluble in strong acids but is quickly and entirely dissolved by ammonium sulphide and is altered by potash. In acute infections it occurs generally in minute grains but in chronic cases coarser particles are found and agglomerations into irregular shaped lumps. Malarial pigment is an iron-containing pigment of haemoglobin which is primarily split up into a proteid globin and the pigment h. ematin from which haemozoin is derived. This pigment is found in no other disease in the circulation though as an extra vascular pathological product a similar pigment is found in schistosomiasis and certain melanotic tumors. However only in the cells of the tumor and never in the blood vessels.

Malarial pigment is most abundant in the splenic vein. In the other blood vessels it is observed in leucocytes but in the splenic vein it is included in large white cells probably identical with the splenic pulp cells. This is apparently due to the fact that the spleen is a special place of destination of the haemozoin laden leucocytes and is also apparently a place of predilection of the parasites.

either *P. vivax* or *falciparum* the reticulocytes were chiefly infected and that as many as 90 to 98 per cent of the infected cells were reticulocytes. Since these observations considerable difference of opinion has been expressed about the matter.

Schüffner and de Graf (1937) observed a greater relative frequency of infection in immature erythrocytes both by *P. vivax* and *P. falciparum*. However DeLangen and Lichtenstein (1936) state that vital staining methods have revealed to them that the parasites choose the older red cells in preference and seldom attack the younger cells.



FIG. 23.—Section of liver in malaria showing (A) phagocytosis of pigment by endothelial and Kupfer cells of the intralobular capillaries of the portal vein.  $\times 215$  (Army Medical Museum photo No. 46952)

More recently Kitchen (1938) has reported that in 2 patients a systematic and comparative study of the parasitic infection of reticulocytes and mature erythrocytes of *P. vivax* showed a definitely greater tendency for the parasite to invade reticulocytes rather than mature erythrocytes even though there was a much larger available number of the latter. A well defined reticulocytosis developed during the latter part of the infections about the time the terminal drop in the parasite density commenced. Spontaneous termination of the attack preceded the return of the immature erythrocytes to normal numbers.

On the other hand in 1939 in the study of 3 *falciparum* and 2 *malariae* infections he found the total number of mature erythrocytes infected with *P. falciparum* constantly exceeded the total number of the parasitized reticulocytes. Infected mature erythrocytes were found on all occasions while infected reticulocytes were observed in only 70 per cent of the examinations. *P. malariae* on all occasions was found in mature erythrocytes in both greater absolute and relative numbers than in reticulocytes. The incidence of infection of the mature erythrocytes by this parasite was extremely low. Van den Bergh and Kovacs (1930) have also reported that the young schizonts of

*P. falciparum* parasitize the erythrocytes of all ages and that the number of adult erythrocytes parasitized are much greater than that of the reticulocytes. Kitchen believes that Wright's stain for vital staining of reticulum in immature erythrocytes by the dry slide method is as satisfactory as brilliant cresyl blue.

The leucocytes, are usually increased during the actual malarial paroxysm but subsequently when the temperature has reached its highest point and toward the end of the attack they become diminished some times to as low as 2000 per cmm. After the malarial attack the leucocyte count may again become normal. However the characteristic picture of chronic malaria is that of a moderate leukopenia with an absolute increase in the monocytes. In fact a leukopenia with a monocytosis of 15 or 20 per cent is suggestive of malarial infection. The greatest increase in monocytes takes place during the apyrexial periods.

**Phagocytosis**—Malarial pigment remnants of degenerating parasites and red blood corpuscles are often taken up by the polymorphonuclear leucocytes and monocytes. Much destruction of the red blood corpuscles both normal and those containing parasites takes place in the spleen. This process of blood destruction is believed to be one of the principal causes of the malarial anaemia.

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By far the greatest part of the phagocytosed pigment in the spleen occurs in the spleen pulp and in the macrophages and larger polyblasts of the pulp but it may also be found in monocytes (large mononuclear cells) and in cells lining the sinuses and even exceptionally in the lymphocytes. It may be present in the arteries of the Malpighian corpuscles but it is only rarely or not at all encountered in the Malpighian corpuscles themselves. Malarial pigment does not occur in the hepatic cells in either acute or chronic malaria. It however is frequently noted in the endothelial phagocytic cells of the liver sinusoids especially in the Kupffer cells of the intralobular capillaries. In these cells it often occurs as irregular masses other instances as finer or coarser grains.

In addition to the haemozoin there is usually found in the organs a considerable amount of yellow or brown pigment *haemosiderin*. This pigment is found not only in the capillaries but also in the parenchyma cells of the liver spleen pancreas kidney bone marrow and the connective tissues. It is found in large quantities in acute malaria in the form of small granules around the central vein of the lobule of the liver gradually diminishing toward the periphery. It is commonly observed in chronic malaria.

*Haemosiderin* which is of erythrocytic origin is however found in increased amounts not only in malaria but also in other diseases associated with excessive destruction of red blood cells. Apparently under this name 2 pigments have been included only one of which contains iron. The other iron free pigment is *haemofuscin*. The iron-containing granules are especially found after active haemolysis and can easily be demonstrated by the blue stain which they take after treatment with potassium ferrocyanide. By this method the haemozoin in which the iron is firmly combined appears black while the haemofuscin remains yellow. In chronic malarial cachexia yellow pigment alone may be present.

*Polycholia*—In some cases of malaria *haemoglobinaemia* becomes marked and then the secretion and flow of bile are correspondingly increased. If this flow of bile is excessive so called bilious symptoms may appear such as bilious vomiting and bilious diarrhoea which are not uncommonly seen in the form of malaria known as bilious remittent fever. In such instances polycholia is a constant and often urgent feature in malaria and gives evidence of the presence of free haemoglobin in the blood. It is not improbable although this point is disputed that the yellow tinge of the skin and sclerae often observed in malaria is due to tainting of the tissue by the liberated haemoglobin and not as is popularly believed to biliousness or cholaemia from bile absorption. Since there is usually a great amount of blood destruction during a malarial attack in many cases an increase in the bilirubin in the blood occurs. Even in cases where there is no apparent jaundice and no trace of bile pigment in the urine the indirect reaction of van den Bergh is sometimes positive and may show a gradual rise during the course of the malarial paroxysm.

The blood sugar is decreased during the course of malarial fever and in the chronic cases there is a marked diminution in the volume of the blood so that at autopsy in the cases of long standing we do not find that congestion of the organs which is usually common in many acute specific infections.

The urine as a rule in infections with *P. vivax* or *P. malariae* shows nothing abnormal though a small amount of albumen and hyalin and granular casts are occasionally observed in severe infections. However in cases of *P. malariae* infection of long duration kidney disease has been frequently noted by MacFie in Nigeria and Gighoh in British Guiana. Lambers reported nephritis in nearly 50 per cent of quartan cases of malaria in Dutch Guiana as compared with 4-5 per cent in *vivax* and *falciparum* infections. Manson Bahr (1940) has applied the term nephrosis to the toxic nephritis which some observers think is more common in quartan infections. Boyd and Proske (1941) found in quartan infections a trace of albumin was definitely associated with a depression in the plasma albumin.

**Morbid Anatomy**—The spleen varies in size color and consistency according to the length of time the infection has persisted and its severity. It is usually more or less enlarged and the surface dark red or chocolate

colored. The cut section is often pigmented. The capsule is not thickened and is easily torn. In rare instances the spleen may not be enlarged and it may be otherwise normal in appearance. In acute cases the parenchyma may be almost diffuent so that the pulp can often be washed away with water. The splenic nodules of a greyish white color frequently stand out with great clearness against the surrounding dark background but in other instances they are not visible in the much swollen pulp. In chronic infections while the spleen is also usually enlarged it is generally firm or of a hard consistency (aguecake). Its color may be greyish or dark slaty or in very severe and long continued infections even black. In some instances it becomes enormously enlarged. In the primary attack much of the enlargement of the spleen may be accounted for by the simple hyperaemia. In older cases the enlargement is due especially to the accumulation of parasites and the hyperplasia of the activated macrophages these apparently being increased in number as well as in size.

The degenerative changes which occur in the Malpighian follicles in acute cases vary from the diminution of lymphoid cells to atrophy and marked necrotic changes. Gross lesions of a more or less degenerative nature which are sometimes present in fatal cases are—haemorrhagic areas in the pulp, thrombi in the arterioles and capillaries of the spleen and various degrees of infarction. Areas of focal necrosis in the spleen pulp and splenic follicles have also frequently been encountered in pernicious cases though there are few reports of their occurrence in chronic ones.

Microscopical examination reveals that the spleen is usually extensively invaded by the parasites and in the case of *P. falciparum* the more mature segmenting forms are generally only seen here. In fatal cases an enormous number of red cells may be parasitized and many monocytes contain the pigment. (See Plate III.)

Microscopic sections often show a diffuse pigmentation except within the Malpighian corpuscles where the haemozoin is found in the surrounding splenic pulp. In both the spleen and the bone marrow the pigment is found in endothelial cells separated from the blood vessels as well as in the endothelial cells of the walls of the blood vessels. Many of the endothelial cells of the sinuses are loaded not only with pigment but with parasites and fragments of cells.

*The Liver*—In severe fatal infections the liver is usually congested more or less chocolate brown in color and somewhat enlarged. In acute fatal cases cloudy swelling associated with varying degrees of vacuolation of the hepatic cell cytoplasm may be observed. In chronic malaria the color is often darker and more slaty and hyperaemia is less marked. In such cases a great increase in the amount of connective tissue throughout the organ may sometimes be present. There however is much doubt as to whether the malarial parasite is capable of bringing about sclerotic changes in the liver. Necrotic foci occasionally occur in the liver in the portal areas. Occasionally they may be sufficiently large to be seen with the naked eye. Most commonly they involve the central zones of the liver lobules.

The endothelial and Kupffer cells are packed with black pigment. The parenchymatous cells of the liver do not contain this pigment but



## PLATE III

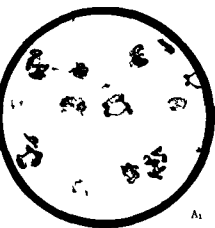
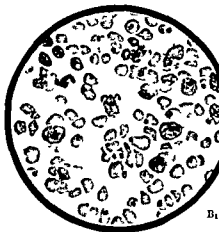
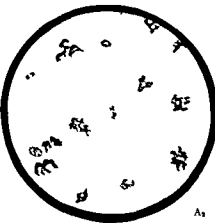
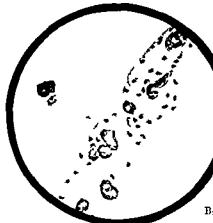
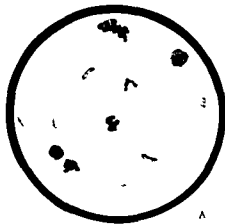
### MALARIA PARASITES

- A Thick Films
- A<sub>1</sub> *Plasmodium vivax* Characteristic for the species in thick films is the presence of the parasite in various stages of development together with the distinctive amoeboid form. The heavily pigmented parasite in the right portion of the field is a gametocyte. The large purplish objects are leucocytes showing the usual distortion seen in thick films.
- A<sub>2</sub> *Plasmodium malariae* Various developmental stages are present as in *P. vivax* but the parasites are smaller, more compact, lack the amoeboid shape, and are heavily pigmented.
- A<sub>3</sub> *Plasmodium falciparum* Only ring forms of the trophozoites are seen. These together with the characteristically shaped gametocytes (crescents) make identification possible.
- B Cerebral malaria. Smears taken postmortem from a fatal case of malignant tertian malaria. (From a case of Lt. J. J. Sapero, Medical Corps, U. S. Navy.)
- B<sub>1</sub> Smear from spleen. Almost one third of the red cells are parasitized with *P. falciparum*. Note the mature schizont in the upper portion of the field and three monocytes containing pigment.
- B<sub>2</sub> Smear from brain. A capillary of the brain distended and blocked with red cells, scarcely one of which has escaped being parasitized. A mature schizont is in the upper left part of the field.
- B<sub>3</sub> Smear from bone marrow. The parasites though less numerous than in the smears from other organs are present in considerable numbers. In the upper part of the field is a melaniferous leucocyte.

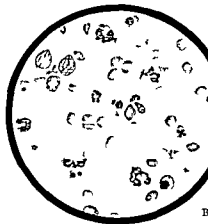
A

## PLATE III

B

A<sub>1</sub>B<sub>1</sub>A<sub>2</sub>B<sub>2</sub>

A



B

(U S N at M I at School)

## MALARIA PARASITES

(A D I)

In Thick Films (A) and in Internal Organs (B)

(T I)

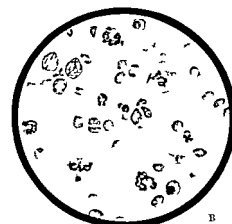
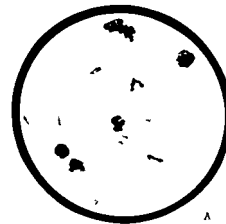
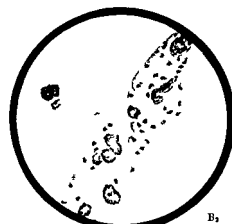
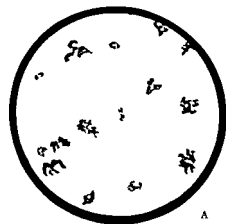
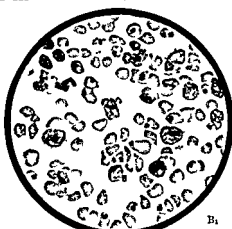
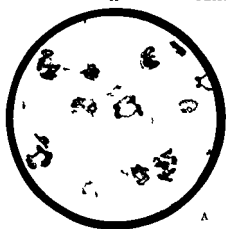
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A

B



(U S N a l M d a l S h o o l)

MALARIA PARASITES

(A D Wall )

In Thick Films (A) and in Internal Organs (B)

(T f p 56)

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le shows no special pathological change However of subtertian malaria Wenyon Dudgeon and Clark fatty degeneration similar to that which occurs in



sulfonamides may be regarded as an exception. However active immunity in a number of acute bacterial infectious diseases not only occurs but terminates the infection and the patient recovers without drug treatment.

The question of the occurrence of immunity in malaria has been extensively studied in recent years not only from the epidemiological standpoint but from experimental inoculations which have been carried on in both man and animals. However in interpreting the results of the inoculations in man which have been carried out by the direct injection of blood containing schizonts or by the injection of sporozoites from mosquitoes or by the bites of infected mosquitoes—many factors regarding the virulence or number of the parasites inoculated the species and conditions of infectivity of the mosquitoes the temperature at which they have been kept and other factors must be taken into consideration in drawing conclusions in regard to the susceptibility of individuals to infection. Much of the work is still in the experimental stage though some definite progress has recently been made.

Immediately after the onset of malarial symptoms a considerable destruction of the parasite occurs. Knowles estimated that a single parasite which might produce 20 merozoites at each successive multiplication if unchecked would have increased in 20 days to a point where there would be about 4 parasites to every blood corpuscle and the patient would have certainly succumbed from such an infection.

In birds two thirds of the parasites produced are destroyed during the stage of development of the disease.

In the experimental studies of birds and monkeys it has been shown that after a varying period of active infection during which parasites are numerous in the blood resistance often develops symptoms subside and parasites can no longer be found in blood films. However it can be shown that in many instances parasites persist in the blood in small numbers (latent malaria) because inoculation of such blood into normal animals produces the disease. As soon as complete cure is effected (after from several months to a year or two) and the blood is no longer infectious the immunity may disappear and the animal can then be reinfected. The immunity which depends upon a persistent latent infection has been termed *premunition*.

The term *premunition* was introduced by Sergeant Parrot and Donatin in 1925 in a study of rickettsial infections. The term is useful with reference to malarial disease especially if it is reserved to imply a stage of acquired immunity in which parasites are scarcely or not at all found on microscopic examination and contrasted with the word *tolerance*—restricted more to the state where the healthy subject is harboring larger numbers of parasites without symptoms of disease.

In man there is considerable evidence to show that acquired immunity may result from repeated infections and that the natives of malarial districts acquire more or less immunity from repeated and persistent infection in childhood. In heavily endemic districts many different



diphtheritic intoxication. In a few instances there have been reports of blocking of the capillaries with parasitized cells. Gaskell has reported the presence of the subtertian parasite among or within the cardiac cells.

A few authors have reported degenerative changes in the *suprarenals*. In a few instances arterial thrombosis, haemorrhages and necrotic areas have been observed. Dudgeon and Clark found the most constant lesion in 30 or 35 acute cases to be the reduction of the fatty lipoids of the cortical layers. It has been suggested that these changes may explain a syndrome occasionally met with in malignant tertian fever characterized by great muscular weakness and low blood pressure.

*Stomach and Intestines*.—In the great majority of cases the mucosa shows little pathological change. However in cases in which gastro intestinal symptoms have been prominent during life lesions in the mucosa are common. Bignami has described punctate haemorrhages in the mucosa of the stomach and large and small intestines. The vessels of the mucosa are sometimes found to be packed with the spore laden parasites and there is sometimes wide spread necrosis of the mucosa. Such lesions have been particularly recorded by Daniels, Koss, Seyfarth, Job, Hitzmann, Craig, Manson Bahr and the editor. Manson Bahr (1930) has emphasized the following changes: (a) intense infection of the mucosal vessels with parasitized cells, (b) necrosis of the epithelium, (c) mucositic infiltration of the tissues subjacent to the necrotic zones, (d) invasion of the necrosed tissues with bacteria. Haemorrhage in the bowel is also sometime encountered. In some insidious cases of malaria are complicated by terminal infections with either bacillary or amoebic dysentery. In other instances the organisms producing these forms of dysentery have not been detected. In some chronic fatal cases of malaria the peritoneum is slate colored and the intestinal mucosa may have a similar appearance. The pigment may be especially confined to the areas of Peyer's patches giving a shaven beard appearance. Meleney (1941) in an important article has discussed the Physiological Pathology of Malaria.

*Pathological Diagnosis*.—The finding of pigmented mononuclears or pigmented parasites in a cross section of a blood vessel is diagnostic of a malarial infection.

However, some malarial manifestations are not uncommon in autopsies in many parts of the tropics and one must be very careful about reporting malaria as the real rather than contributing cause of death.

There is usually a marked increase in the large mononuclear cells in malaria and if this is noted together with a leukopenia it is very suggestive. Melaniferous leucocytes occur in malaria only. Their presence is therefore diagnostic but usually the parasites are more abundant than pigmented leucocytes and the diagnosis is usually more evident through the detection of the parasites than through the finding of pigmented leucocytes.

*Immunity*.—The question of immunity in malaria is a difficult one to understand. In protozoal infections there is little definite evidence of the production of an active immunity which corresponds to that which occurs in many bacterial infections. Another point of distinction is that certain protozoal diseases are markedly influenced by drugs as malaria by quinine, yaws and syphilis by salvarsan and leishmaniasis by organic antimony compounds. This obviously is quite in contrast to the conditions in the treatment of many bacterial infections which are influenced specifically generally very slightly by drugs and there are practically no specific drugs for bacterial diseases although the action of some of the

Of 72 negroes 56 became infected after primary mosquito or subsequent reinoculation (with *P. falciparum*). However among 60 patients (8 white) successfully inoculated in this manner there were only 45 in whom inoculation took on the first application of the mosquito. James also has reported the case of a West Indian negro who had passed most of his life in Europe and so far as could be ascertained never had been infected with malaria. Many unsuccessful attempts were made to inoculate him with malaria. He showed a complete absence of any detectable infection after inoculation with 2 strains of *P. falciparum* and one of *P. knowlesi* both from infected monkeys and infected men. Heavy doses of quartan blood produced only a mild and transient infection with *P. malariae*. However a mild infection was said to be produced by 2 injections of blood containing *P. ovale*.

Midam and Kusch (1938) have also demonstrated the fact that negroes are less susceptible to *P. knowlesi* than whites. Susceptibility of whites to this parasite seemed to be universal from inoculations made into 29 white persons but in 6 negroes there was almost no response. Blood specimens from 4 of the negroes showed parasites in the thick film only and the remaining 2 were negative throughout. The 2 negative cases however were noted to have experienced subclinical attacks since inoculation of their blood into normal monkeys revealed the presence of parasites for as long as 3 weeks following their inoculation.

Wilson (1939) has drawn attention to the apparent controlling effect upon malarial infection during the breast feeding period. Schwetz (1933) in the Congo, Clark (1937) in America and Barber (1937) in Macedonia have all remarked on this phenomena. As yet however there is no definite evidence of any immunity or tolerance to malaria in newly born infants dependent upon a specific antibody absorbed during lactation. It seems more probable that a passive transmission of immunity through the placenta might occur. Sinton (1939) suggests that immunity might perhaps be acquired *in utero* by the child as the result of the stimulation of toxins absorbed from the maternal circulation and that any such specific immunity is probably diaplacental and not colostral.

*Demonstration of Antibodies in Malaria*—Attempts have also been made to demonstrate the presence of specific antibodies in the host during a course of an infection due to malarial parasites. In avian malaria for example the recovery from an initial attack sometimes occurs with such rapidity that it has been referred to as a crisis. However in the literature one finds conflicting reports concerning the protective power of serum taken from man or other animals suffering from chronic malaria.

Coggeshall and Kumm (1937) point out that since it is known that from the onset of the infection macrophages of the infected blood are constantly phagocytizing parasites the marked decrease in their numbers at the time of recovery could probably best be explained on the basis of the presence of opsonizing or humoral immune substances. Furthermore the fact that an animal after recovering from a malarial infection is usually immune to reinfection with the homologous parasite would tend to suggest that there are specific protective substances present in the serum of such an animal. Hence a number of observers have inferred that specific humoral immune substances to malarial parasites are present in the serum of the host during chronic infection but it has been supposed that these antibodies were present in the serum in such low concentration that their presence could not be demonstrated by ordinary protective test methods. Coggeshall and Kumm however have shown that infection in these monkeys with *P. knowlesi* which (if untreated) is almost invariably fatal in these animals can be made chronic by the early administration of antimalarial drugs. The animals will

surveys have shown that over 90 per cent of the young children have malarial parasites in their blood, while among the adults of such districts the rate of infection is usually much lower. In such regions one finds many young children playing about apparently well who nevertheless have parasites in their blood. Many of these children have evidently acquired a resistance, or tolerance, against the infection, since the malarial parasite no longer gives rise in them to acute symptoms of disease. This resistance or tolerance is often marked in adults in such infected districts and sometimes only in certain instances is a latent infection revealed as when the individual is so exposed to great fatigue or hardship or cold, that a malarial paroxysm is precipitated with the resistance being at least temporarily lost. It has also been repeatedly shown in the treatment of general paralysis by induced malaria that a patient frequently becomes immune to the strain of plasmodium employed though he can sometimes be reinfectd with another strain of the same species.

Boyd and Mathews (1939) have shown that homologous immunity to a strain of *P. vivax* may prevent a clinical attack for as long as 6 years though after 3 years the patient was given a heavy reinoculation with the same strain he first received. Boyd Thomas and Kitchen have also found that reinoculation of a patient who had recovered from a *P. falciparum* infection did not result in a second clinical attack although plasmodia could be found in the peripheral blood during this latent stage for as long as 4 months. On the other hand they found that in a patient who had recovered from a *P. falciparum* infection reinoculation with a different strain of the same species resulted in a clinical attack of malaria. They point out that the immunity produced by infection with the plasmodia is largely homologous and that the presence or absence of a latent infection appears to exert little if any influence on the heterologous inoculation. In other experiments they showed that recent recovery from either a *vivax* or *falciparum* infection is no obstacle to successful reinoculation with the other species of parasite hence there is absence of cross immunity between *vivax* and *falciparum* infections.

**Racial Immunity**—There is relatively little evidence of natural immunity to malaria in the white race although occasional individuals appear to be much less liable to contract the disease than others. However Van Loon and Kirchner (1924) have shown that a high degree of tolerance, if not actual immunity exists with certain of the natives of the Dutch East Indies. Properly controlled inoculations were negative with them. It also seems evident that negroes are frequently less susceptible to experimental infection with malaria than whites and that there is apparently a natural or racial insusceptibility in them. There is much evidence to show that this is not an acquired immunity but a racial factor.

Thus several investigators have demonstrated the special resistance of negroes with reference to the inoculation of *P. vivax*. Boyd and Kitchen (1937) have however shown that some negroes exhibit less resistance to infection with *P. falciparum* than they do to *P. vivax* inoculation.

immunity have also pointed out that the reticulo-endothelial system through phagocytosis is intensively active in overcoming malarial infection.

The sensitization of the phagocytes which has been regarded especially as the basis for acquired immunity to malaria is apparently a delicate mechanism easily upset as is suggested by the frequency of relapses in malaria when the susceptibility of the individual to infection is heightened by shock, exhaustion, change of climate or alcoholic excess. Obviously the immunity or resistance cannot be explained entirely by phagocytic activity and the process is apparently associated with the production of antibodies and protective substances.

Chandler (1940) believes that the persistence of *lax* and malarial infection in the body over a period of several years and often characterized by relapses leads to a higher ultimate degree of immunity in these than results in *falciparum* infection.

Boyd and Kitchen (1943) have found that recovery from an attack of *lax* malaria results in a very potent immunity to the homologous strain. Early reinoculations were sometimes followed by the return of subclinical parasitemia. Later inoculations may not be followed by any parasitemia when the patient may be considered as hyperimmune.

**Wassermann and Kahn Reactions**—The occurrence of the Wassermann and Kahn reactions with the blood of malarial cases is of some interest in the discussion of immunity in malaria. However it should be emphasized that the occurrence of the Wassermann reaction in syphilis should not be regarded as an index of immunity. Although in earlier years it was thought that this reaction as applied to syphilis was a true antigen-antibody reaction, now it is recognized that the body in a syphilitic serum which reacts with the antigen is not an antibody but a lipoidophilic substance which has the property of linking complement to the lipoidal antigen. It has long been known that the Wassermann and Kahn reactions occur in many cases of malaria where syphilis (and yaws) are excluded. Nevertheless many contradictory opinions have been expressed with reference to the occurrence in malaria of non-specific positive reactions with both the complement fixation (Wassermann) and the precipitation or flocculation test (Kahn) for syphilis.

In the United States Folmer (1929) who has been an authority regarding the specificity of these reactions stated that in his experience malaria and many other infections had no influence *per se* on the Wassermann reaction although the serums in acute febrile diseases might become somewhat more anticomplementary than usual. Later he reported 62 negative reactions in non-syphilitic patients with tertian and aestivo autumnal malaria and stated that it was his confirmed belief that the serums of non-syphilitic malarial patients did not yield falsely positive reactions with his new method even when the blood was drawn just before or after a paroxysm of chills and fever. However Cumming and his associates in the United States Public Health Service later carried on an extensive study of the value of the sero-diagnostic tests for syphilis. Specimens of serums from non-syphilitic persons but with malaria or other diseases were distributed to a number of serologists who performed Wassermann or Kahn tests. Folmer's laboratory reported positive Wassermann reactions for 13.4 per cent of the specimens obtained from patients with malaria. In the four laboratories in which the complement fixation tests were done the percentage of positive results for the malaria patients ranged from 8.6 to 20.6. Also Hazen (1938) obtained positive results in 8 per cent of the tests on 266 patients with malaria, presumably non-syphilitic. He noted a higher proportion of positive reactions among females. Taussig and Orgel (1937) who employed the Kahn test in malaria summarized the non-specific results of a number of observers as varying from 4.0 to 80 per cent. While Kurth

then harbor a chronic infection for an indefinite period. The serum taken from such monkeys with chronic infection and injected into monkeys suffering from an acute attack is found to have a definite depressing effect on the course of the experimental disease. In some instances death was prevented and an acute infection changed into the chronic form. They conclude that protective antibodies are produced in the serum of monkeys during experimental malaria infection, however the nature and mode of action of these protective substances and those in chronic malarial infections is at present not known.

Eaton (1938) has reported a specific agglutination of *P. knowlesi* detectable both by macroscopic and by microscopic methods. Agglutinins for *P. knowlesi* were found to appear in the sera of monkeys between 15 and 45 days after the onset of the infection and became progressively stronger as the malarial infection gradually subsided. Agglutinins persisted in the sera of chronically infected animals for a year or longer. The sera of animals which had been repeatedly superinfected agglutinated the parasites at dilutions as high as 1 to 1000.

Sera from normal monkeys, from monkeys acutely ill, and from monkeys chronically infected with a different species of malarial parasite *P. vivax* did not agglutinate *P. knowlesi*. Immune serum agglutinates mature intracellular or extra cellular parasites but does not agglutinate unparasitized cells or cells containing immature parasites. With reference to this specific agglutination of *P. knowlesi* by immune serum it is suggested that a specific sensitization of the parasites may occur *in vivo* and that this renders them more susceptible to phagocytosis by macrophages of the spleen and by other phagocytic cells. The appearance of agglutinins in the sera of monkeys after the recovery from the acute phase of the infection with *P. knowlesi* also suggests that the relative immunity of these animals is associated with the presence of sensitizing antibodies in the blood stream.

Complement fixation is reported by Eaton and Coggeshall (1936) in human malaria with an antigen prepared from the monkey parasite *P. knowlesi*. The most sensitive and specific malarial antigen was prepared from dried parasitized red cells of monkeys dying with the infection, the cells being extracted with saline after freezing and thawing. This *P. knowlesi* antigen is reported to give strong complementary fixation with malarial sera from human beings infected with *P. knowlesi*, *P. vivax* or *P. falciparum*. The titer of the complement fixing antibodies reached a maximum about one month after the beginning of the acute infection. At this time all of the *P. knowlesi* sera tested were positive. This complement fixation reaction in malaria was group specific rather than species-specific. Sera from patients infected with *P. vivax* or *P. falciparum* react in the same way with *P. knowlesi* antigen as the homologous sera. In a subsequent article Eaton (1939) reports the presence of a soluble malarial antigen which fixes complement present in the serum of monkeys infected with *P. knowlesi*. (See also Coggeshall: Immunity in Malaria Medicine 27: 87, 1943.)

German in Simmons and Gentsko v (1944) discusses the value of the reaction in the diagnosis of human malaria and points out that the test becomes positive approximately 2 weeks after the initial paroxysm and persists for about 5 months after circulating parasites disappear from the peripheral blood. Relapses cause a rise in titer indicating that circulating parasites are a source of antigen and thus are necessary to maintain the titer at a significant level.

Especially from experiments upon animals is there evidence that immunity or resistance in malaria develops particularly in connection with a proliferation and increased activity of the reticulo endothelial cells, especially of the spleen and also of the liver and bone marrow which engulf and destroy the infected red cells as well as the free parasites. This stimulation is possibly a manifestation of a true hypersensitive reaction to the foreign protein of the parasite as it disappears when the parasites are completely eliminated. Splenectomy greatly increases the susceptibility of monkeys to malaria as does blockade of the reticulo endothelial system in birds. Taliaferro (1941) and others who have suggested the presence of a cytolytic antibody as an important the

the temperature makes a critical fall to normal or subnormal readings such fevers are frequently designated *intermittent fevers*. These so called benign infections rarely exhibit pernicious manifestations. They may however as with the more dangerous aestivo autumnal parasite *P. falciparum* lead to the production of malarial cachexia in which the clinical manifestations are similar whether produced by a benign or malignant species.

(2) *Those in which the succession of cold hot and sweating stages is lacking*. There is not the frank well defined chill of the former group so that the term dumb chill is frequently applied. With the possible exception of the first paroxysm the temperature tends to remain well above normal giving a continuous or remittent type of fever instead of the intermittent temperature curve of the benign infections. The designation *remittent fever* is often applied to such fevers. Clinically there may be a resemblance to typhoid fever. In such malarial fevers small hair like ring parasites and crescentic sexual forms are usually found. There are many clinical designations for this type of malarial fever of which the best recognized are malignant tertian subtertian aestivo autumnal and tropical. It is pre eminently the malarial fever of the tropics and from its appearance in temperate climates chiefly in the late summer and through the autumn months it received from the Italians the designation aestivo autumnal. Such fevers were called *subintrant* by Torti because the succeeding paroxysm set in before the completion of the long continued preceding one. The designation *malignant tertian* is perhaps preferable as indicating the greater seriousness of this type of malaria. It is in infections with this species that the symptoms of blackwater fever may occur and in which malarial cachexia is encountered.

**Incubation Period**—The incubation period varies considerably as is evident from the many different reports in the literature. The susceptibility of different hosts is probably of considerable influence in determining it. From experimental inoculations James found that the mean period after mosquito bite was about 14 days but that when caused by blood inoculation it was in the neighborhood of 11 days.

Milam and Coggeshall (1938) found that in blood inoculations of white patients with *P. knowlesi* parasites were sometimes seen in the blood smears as early as the third day. In a series of 181 experimental successful inoculations with infected mosquitoes Boyd and Kitchen (1938-39) found with *P. vivax* an incubation period (as judged from the time of infection up to the time parasites were demonstrated in the blood) which varied between 8 and 23 days. It has been found that there is sometimes a tendency for the incubation period to become shorter especially in subtertian malaria and for the attacks to be more severe when the number of bites is increased. In a small series of experiments performed with *P. falciparum* Boyd found the incubation period was as in the case of *P. vivax* not less than 8 days. In naturally acquired infection with *P. malaria* Boyd (1940) found the incubation period often exceeded 4 weeks. Greig (1939) also observed an average incubation period with *P. malaria* of 14 days by direct inoculation of the blood. However latency is very characteristic of malaria and the onset of clinical manifestations with all forms of infection may be postponed for several weeks or months. Protracted latent periods of development have been occasionally reported by various observers of 10 months or longer.

in Guatemala and Wilson and Levin also reported falsely positive reactions from malarial blood

On the other hand Lloyd and Mitra (1932) and Needles, Menk, Greer and Heimann in earlier years believed that malaria did not cause positive serological reactions with modern technique. Saunders and Turner (1935) also concluded that malaria does not cause fixation of complement with the method they used but that it might raise a low titer reagin to the complement fixation threshold.

By far the most important contribution to the subject that has appeared in more recent years is that of Kitchen (1939) and his associates in which systematic studies of the Wassermann and Kahn reactions were made before, during and after 25 naturally induced attacks of malaria in non syphilitic patients. Positive reactions were obtained in every case in which malaria developed clinically.

Two inoculation cases showed no positive results from the complement fixation tests and two others showed no positive Kahn reactions. There was evidence of malaria provoking positive serologic reaction and a considerably increased cell count in the cerebrospinal fluid. Seventy two per cent of the positive reactions made their first appearance during the third and fourth weeks following inoculation. However the first positive reactions were observed both before and after the period of clinical activity in a few instances but in 63 per cent of the attacks they occurred before the first two weeks of the febrile period. The positive reactions exceeded 3 weeks in 60 per cent of the cases and extended beyond 4 weeks in 48 per cent. During this time 320 positive and 307 negative serologic reactions were obtained.

There was a tendency for *m. ax* infections to induce a greater proportion of positive serologic results than *falciparum* infections and the positive reactions were higher among females with malaria than among males, and among persons up to 35 years of age than among those over that age. The percentage of positive reactions was higher during the period from 15 to 21 days after the last previous paroxysm. There was no clinical evidence of coincidental syphilis in any of the cases. Also the negative results obtained both prior and subsequent to the positive results demonstrated the reactions were due to malarial infection and not in any way to syphilis.

The results obviously emphasize again most conclusively the fact that the serological test for syphilis (Wassermann reaction) is not a specific reaction and that the diagnosis of syphilis should not be made from the serologic test alone. Recent experiments in which positive Wassermann or Kahn tests have been reported with other diseases also emphasize these facts.

If a positive Wassermann reaction or Kahn test is regarded as necessarily indicative of syphilitic infection, grave mistakes will undoubtedly occur from time to time and may wrongly influence the diagnosis and treatment of malaria.

Among the tropical diseases in which these reactions have been especially recorded positive besides malaria and yaws are leprosy, trypanosomiasis and relapsing fever (as well as a number of febrile infections in temperate climates).

### SYMPTOMATOLOGY

Clinically we have 2 types of malarial paroxysms. (1) Those presenting a cold stage followed by a hot stage with a terminal sweating stage. Such attacks are brought about by the benign infections which include the benign tertian and the quartan. Owing to the fact that in such paroxysms

jaws give way to actual chill with shaking body and chattering teeth face pinched and bluish and cutis anserina

The pulse is frequent small and of rather high tension there is increased frequency of urination and nausea and vomiting may be present Notwithstanding the fact that the rectal temperature is steadily rising five or six degrees during this cold stage there is a desire on the part of the patient to cover himself with all the wraps obtainable The cold stage which usually lasts from 20 to 60 minutes is succeeded by the hot stage At first there is a feeling of slight relief from the misery of the chill but this is soon lost sight of in the increasing headache and feeling of intense heat The previously welcome blankets are cast aside The face now becomes flushed the eyes shining and the pulse more full

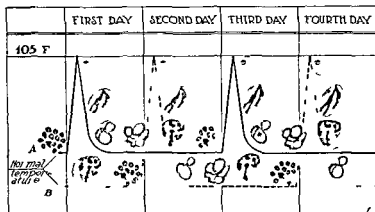


FIG 24.—Diagram of the temperature chart of a double tertian malarial fever showing the successive slopes of two generations of parasites causing the febrile stages. The solid line A shows the development of the generation of parasites first introduced and the dotted line B the cycle of the generation introduced later on.

Epigastric discomfort nausea and vomiting are apt to become more prominent in this stage The patient often complains of a throbbing headache It is at this time that he may become slightly delirious A sense of tension or even pain may be experienced in the region of the spleen which organ will be found tender even if not already palpable Herpes about the nose and lips is almost as common as in lobar pneumonia An attending bronchitis is not uncommon

The fever remains high from 103 to 106 F and continues so elevated for from 1 to 4 hours to be succeeded by the sweating stage In this the dry skin becomes moist and perspiration breaks out first on the forehead to be followed by a more or less marked profuse sweating of the entire body The pulse becomes slower the temperature falls rapidly and the patient falls asleep to awake slightly exhausted but feeling well

This feeling of well being continues during the fever free day which is often referred to by a patient as my good day



Greig (1939) in 144 cases of malaria induced by direct inoculation of infected benign tertian blood found the incubation varied between 2-24 days. The variation indicated differences in susceptibility to infection. The shortest incubation periods were in cases inoculated intravenously.

In addition to the different susceptibility of individuals the number and condition of the sporozoites have been observed to play a role in determining the duration of the incubation period. Barber (1936) found that in Macedonia *A. superpictus* was relatively a poor vector of malaria as compared with *A. elutus* which he thought was in part explained by the fact that the sporozoites of infected *superpictus* dissected during the summer months frequently showed evidence of degeneration which was not true in *A. elutus*.

**Prodromata**—There may be prodromata of the nature of malaise, vague muscular pains, headache and anorexia, possibly showing a periodicity in their appearance or intensity. It is only when a sufficient number of parasites sporulate simultaneously and pour out into the circulation sufficient toxic material to cause a well marked paroxysm that such occurs—with less poison we may only have vague suggestions of an attack of ague. In a large proportion of cases there are no prodromata; they begin with a sudden onset. Malarial paroxysms show a preference for the forenoon or at any rate tend to occur in the early afternoon rather than in the evening. Farley and Muhlenz have noted that in infections with *P. ovale* the fever comes on rather in the evening or at night.

**Multiple Infections**—Quotidian paroxysms may perhaps be produced at times by infected mosquitoes biting on successive nights so that one crop will mature and sporulate twenty-four hours before the second. However in the first attack of (tertian) malaria, whether spontaneously acquired or experimentally produced by one bite of a single infected mosquito, the temperature during the first few days of fever is continued or irregularly remittent. Then as the parasites become assembled into groups, quotidian paroxysms of intermittent fever occur, double tertian infection. Later one group of parasites often dies out and the fever becomes single tertian. In relapses the fever is intermittent and tertian from the onset. In quartan malaria the infection is usually single but double and triple infections may occur. In aestivo-autumnal infection the paroxysms if present may be quotidian or tertian but there may be a continued or remittent fever. Anticipation and retardation in the sporulation may cause a very protracted paroxysm lasting 18 to 36 hours; this tends to give a continued or remittent fever instead of the characteristic intermittent type.

**Mixed infection** is a term applied to the simultaneous presence of two or more species of parasites in the same individual. Mixed infections with malignant tertian and benign tertian are the most common; next malignant tertian and quartan, while cases with benign tertian and quartan are even rarer. All 3 species have been reported in the same individual. In double infections of malignant tertian with one of the other species the former parasites are often absent or undetected in ordinary blood films and yet may be transmitted by inoculation. This is of great practical importance in therapeutic inoculations with malaria since such double infections have a grave prognosis (a mortality of 50 per cent and higher has been reported).

**Clinical Types** *A Typical Benign Tertian or Quartan Paroxysm*—(Other than for the difference in periodicity the paroxysms of these 2 malarial infections are alike.)

The ague attack generally commences with malaise and slight headache, frequently accompanied by yawning and stretching. Chilly sensations radiating from the spinal column to the extremities and the

jaws give way to actual chill with shaking body and chattering teeth face pinched and bluish and cutis anserina.

The pulse is frequent small and of rather high tension there is increased frequency of urination and nausea and vomiting may be present. Notwithstanding the fact that the rectal temperature is steadily rising five or six degrees during this cold stage there is a desire on the part of the patient to cover himself with all the wraps obtainable. The cold stage which usually lasts from 20 to 60 minutes is succeeded by the hot stage. At first there is a feeling of slight relief from the misery of the chill but this is soon lost sight of in the increasing headache and feeling of intense heat. The previously welcome blankets are cast aside. The face now becomes flushed the eyes shining and the pulse more full

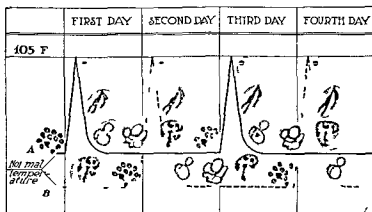


FIG 24.—Diagram of the development of two generations of parasite during the course of a double tertian malarial fever. The solid line A shows the development of the generation first introduced and the dotted line B the yield of the generation introduced later.

Epigastric discomfort nausea and vomiting are apt to become more prominent in this stage. The patient often complains of a throbbing headache. It is at this time that he may become slightly delirious. A sense of tension or even pain may be experienced in the region of the spleen which organ will be found tender even if not already palpable. Herpes about the nose and lips is almost as common as in lobar pneumonia. An attending bronchitis is not uncommon.

The fever remains high from 105 to 106 F and continues so elevated for from 1 to 4 hours to be succeeded by the sweating stage. In this the dry skin becomes moist and perspiration breaks out first on the forehead to be followed by a more or less marked profuse sweating of the entire body. The pulse becomes slower the temperature falls rapidly and the patient falls asleep to awake slightly exhausted but feeling well.

This feeling of well being continues during the fever free day which is often referred to by a patient as my good day.

The sweating stage lasts usually about 2 or 3 hours so that the entire paroxysm of cold hot and sweating stages occupies approximately 8 to 12 hours. While most cases of the benign infections show the typical stages yet we meet cases where the cold and sweating ones are absent or but slightly marked. Blood examination will show the parasites of the benign infections to be in the peripheral circulation during the entire apyrexial period. During the paroxysm we have a moderate leucocytosis and during the afebrile period a leukopenia with an increased percentage of

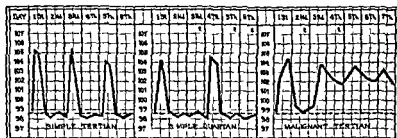


FIG. 25 —Typical fever charts of the 3 types of malaria

large mononuclears. Billet found that quartan paroxysms may differ from benign tertian ones by their showing a less abrupt fever rise and a more rapid fall of temperature with a shorter duration of the paroxysm 4 or 5 hours as against 8 to 12 hours for benign tertian. DeLangen believes the quartan species more benign than *vivax*. However this is not invariable. Different results may be obtained for example with quartan strains of different virulence or according to the number and condition of sporozoites inoculated. Boyd found in therapeutical inoculations that the quartan paroxysm required more time than the tertian. He also found that the disease produced by the quartan parasite was more severe than with the tertian despite the slower evolution of the quartan. It is commonly reported that the quartan infection is the most resistant and persistent infection.

**4 Malignant Tertian Paroxysm**—The characteristic features of the paroxysm are slight chilliness instead of a frank chill, prolonged and intensified hot stage, lack of marked terminal sweating and a tendency to exhibit a continuous or at least remittent fever curve instead of the distinct intermittence with an apyrexial period of the benign infections. During the period of the remittance the patient fails to experience a sense of well being. He is sick and does not have a well day. The temperature of a malignant tertian paroxysm may fall to normal during the first attack but succeeding attacks only show the tertian periodicity by an exacerbation of the more or less continuous fever. In these cases the temperature rise is gradual rather than abrupt and the fall rather by lysis than crisis. The paroxysm often lasts from 20 to 36 hours instead of 10 hours.

To explain the continuous type of fever it is often stated that anticipation and retardation are characteristic of malignant tertian infections. This simply means that the new paroxysm tends to come on before the period of 48 hours has expired and having appeared tends to delay its termination. At any rate there is an extreme irregularity in the course of the paroxysm. These attacks are often termed dumb chills and are greatly dreaded by the patient. The onset is insidious, occurring as a rule in the forenoon or early afternoon, with rarely a chill but only chilly sensations. The headache and backache are severe, the face is flushed, the pulse quickened and the thirst urgent. Not infrequently we find an initial bronchitis. The patient feels more prostrated and ill than does one in a benign paroxysm and there is a distinct tendency to mental confusion or delirium. Nausea and vomiting may be prominent features of an attack. At times an apathetic state may suggest typhoid fever and in earlier years was often confused with it. In these malignant malarial attacks the spleen generally is palpable and very tender. There also may be a sensation of weight in the region of the liver.

In a blood examination one may find only the young ring forms which begin to appear a few hours after the onset of the paroxysm. The rings may be observed to broaden but prior to that development in which pigment would appear in the ring the parasite-containing red cell is generally arrested in the capillaries of the spleen or other organs. The finding of young ring forms while fever continues is suggestive of an early malignant tertian infection. In the absence of quinine administration the finding of parasites is to be expected in benign tertian and quartan infections but with the tropical parasite a smear may fail to show any organisms where a few hours previously a blood examination might have shown a large percentage of infected red cells in many fields of the microscope.

Subtertian malaria may assume different clinical types of disease.

In the *cerebral forms* the parasites are found in enormous numbers in the brain capillaries. Clinically the predominant features may be hyperpyrexia, delirium, coma, psychoses of various types which may simulate acute alcoholism, convulsions (in children), signs of meningeal irritation, amblyopia or apoplectic phenomena (hemiplegia, aphasia, etc.). In the *algid forms* the parasites tend to accumulate in the capillaries of the gastrointestinal tract and abdominal organs. There is usually profound prostration with a tendency to fatal syncope and extreme coldness of the skin with a high internal temperature. Several clinical types may be distinguished: gastric with incessant vomiting, choleraic dysenteric with blood in the watery stools, haemorrhagic or purpuric. A grave haemolytic anaemia may develop with great rapidity. The majority of cases of malignant tertian malaria, justly termed pernicious, are cerebral or algid forms. The pernicious manifestations have been explained by (1) the very large number of red cells infected and destroyed by the malarial parasites, (2) the throwing off at the time of sporulation of the merozoite of a large amount of toxic material owing to the presence of such a large number of disintegrating merozoites and (3) the plugging of the capillaries of important internal organs by adult parasites. This may arise as the result of (a) the sporulating parasites acting as emboli, being too large to pass the lumen of the capillary, (b) from degenerative changes or distension by red cells and pigment of the endothelial cells lining the capillaries, or (c) as the result of an ovoid shape on the part of the *malignant tertian parasite* there is an inability to pass through capillaries which the flattened benign parasites can do by infolding (Bass) or (d) resulting from the tendency of malignant tertian parasites to agglutinate in the capillaries and tissues.

Knusly et al. (1941) and Lack (1942) have studied by means of quartz rod transillumination the blood vessels of monkeys infected with *Plasmodium falciparum*. In the intravascular agglutination of parasitized red cells in malaria a layer of fibrin or similar substance is deposited on the cells causing them to stick together but not to the end

Helpern (1934) and Most (1940) have found cerebral involvement particularly common in drug addicts who became infected by direct inoculation of infected blood. Such cases often result fatally.

*Algid Manifestations of Pernicious Malaria*—Severe diarrhoea unaccompanied by fever and often ending fatally which in its intensity and rapid course resembles true cholera has long been recognized as an algid form of pernicious malaria.

The choleraic symptoms may develop unaccompanied by rigor or any of the more familiar signs of subacute malaria or they may follow an acute attack. In such cases there is often a small thread like pulse and a cold clammy skin. The respiration is often slow and shallow and the voice weak. As in cholera the dehydration consequent upon the excessive diarrhoea may lead to cramps, pinched features and fingers, suppression of urine and collapse. Cases of this nature have been especially reported in India. Manson Bahr (1939) has also called attention to their occurrence in Palestine and Salonica during the World War, several of which were fatal. He emphasizes the necessity of recognizing the gastro-intestinal symptoms as indicative of the severe subtertian malaria infection which may eventually end in coma or death.

It has been customary to classify these cases with vomiting, diarrhoea, painful cramps of the legs and scanty or suppressed urine as choleraic in type. However other cases of algid malaria may show blood or mucus in the stools and have marked abdominal pain and these have been termed dysenteric. Some resembling more the dysenteric type at times show only a diarrhoea and at other times the presence of blood. The dysenteric type is more common. The question may arise as to whether the symptoms are due to the recrudescence of a previously existing dysentery of other than malarial origin or to the development of such a form of dysentery through the lowered resistance of the patient from the malarial infection.

It seems clear that malaria may light up a bacillary infection or that this disease may light up a latent malaria. Biggam however has found in drug addicts where infection resulted from direct inoculation of malarial blood that the symptoms might be of the dysenteric type.

*Malarial Dysentery*—In fatal cases of malaria with dysenteric symptoms the intestines at autopsy may be congested and dark red in color or have a mottled appearance as in catarrhal dysentery while the contents may be blood stained and contain mucus. Nocht and Mayer emphasize that in such forms of malarial infection not only sanguinous mucus discharges may occur but also intestinal haemorrhage. The malarial parasites and pigment together with swollen endothelial cells may form veritable thrombi and occlude the vessels in the intestinal mucosa. The epithelial cells subsequently become necrotic. Whether this form of dysentery owes its origin to malarial intoxication in especially susceptible individuals is not clear. The disturbed circulation in the capillaries due to the presence of the parasites also predisposes to secondary infection and both bacillary and amoebic dysentery have been found in autopsy in cases of severe malarial infection. Such cases were seen during the Great War in Salonica and Palestine. In other instances of malarial dysentery neither *Endamoeba histolytica* nor *Bacillus dysenteriae* were found. On 2 occasions with intestinal symptoms Manson Bahr made the diagnosis of subtertian infection by demonstrating the ring forms of the parasite in film preparations from the stool.

Stoll in his studies of malaria reported 5 algid cases of dysenteric type but not one of choleraic. When epistaxis and haemorrhages from the intestines or stomach are marked features of an attack the cases have been termed haemorrhagic. Cases have been reported where the excessive sporulation was apparently taking place in the pancreas giving the symptomatology of acute haemorrhagic pancreatitis.

*Bilious Remittent Fever*—This is the most common and the least dangerous of the pernicious manifestations but tends rapidly to produce malarial cachexia. Slight jaundice and bilious vomiting may appear

in the course of an ordinary malignant tertian paroxysm and only severe types with fatal tendency should be classed as pernicious. It sets in with marked nausea followed by vomiting and bile rich stools. Jaundice shows itself by the second day earlier than in yellow fever but much later than the rapidly appearing jaundice of blackwater fever. The urine often shows bile pigment and a yellow foam and bilirubin may be present in the blood. Epigastric distress and liver tenderness are marked features and there may even be gastric haemorrhage.

*Pneumonic and Cardiac Types*—Other recognized types are the so called pneumonic type in which with the symptoms of a broncho pneumonia we find an element of periodicity and a response to quinine. Again usually in elevated regions dilatation of the right heart and death have been noted as occurring in cardiac types of pernicious malaria. Another type is that in which the sweating stage is excessive the so-called diaphoretic type. These cases may result in collapse and such a termination may be syncope in character.

**Relapses**—Relapses are characteristic features of malarial disease in which the clinical symptoms return and the parasites which may have been absent from the blood are found again sometimes in large numbers.

The term *recrudescence* is sometimes used for attacks occurring within 2 months of the original infection and *recurrences* for attacks occurring after an interval of 7 or 8 months. The late recurrences are a characteristic feature of benign tertian malaria but do not usually occur in malignant tertian. Gametocytes are much more numerous during a recurrence than in the primary attack (James 1936). Relapses are highly characteristic of all types of malarial infection being most marked in quartan and least in malignant tertian.

Relapses may occur without obvious cause but are apt to follow any condition which lowers the general resistance of the body. Among these may be mentioned exposure to cold or wet to intense sunlight excessive fatigue alcoholic dietetic or venereal excesses an intercurrent illness a serious accident (fracture) a surgical operation and child birth. Persons returning home from the tropics often experience relapses as they approach the cooler climate of the temperate zone. It has been well stated that the old resident of the tropics owes his condition of health rather to education than to acclimatization—experience has taught him discretion.

It is now believed that relapses are due to failure of the defensive forces of the body to restrict the multiplication of the parasites to negligible proportions as they do during the latent stages of the infection (Ross Bignami). There is no proof of the existence of special resistant asexual forms. Schaudinn's theory that female gametocytes may undergo parthenogenetic multiplication has been disproved completely. Attempts to transmit malaria by inoculation of blood containing only gametocytes have been uniformly unsuccessful.

*Latent malaria* is a term applied to persisting infections which give rise to no clinical symptoms and in which parasites usually cannot be demonstrated except by inoculation of large amounts of blood into other individuals as in the treatment of paresis.

The persistence of a malarial infection in the absence of clinical and to a great extent of laboratory manifestations is sometimes shown by the occurrence of relapses so that the section treating of malarial relapses applies also to this paragraph. In addition to the factors influencing relapses already mentioned it should be noted there is a particular tendency for a latent malaria to develop activity following surgical operations and childbirth. In untreated latent cases healing of surgical operations may be delayed.

The importance of examining placental smears when obtainable for evidence of a latent malarial infection has been noted. Persons returning to a cool climate from the tropics who may not have shown evidence of active malaria for months may come down with a paroxysm upon encountering cool weather (refrigeration). Latency may be complete or there may be vague manifestations of ill health such as anorexia, malaise, irritability, headaches, anaemia and alimentary tract disturbances or some form of neuralgia. Not infrequently tropical residents without symptoms may show crescents in their blood and such cases are of prime importance in connection with infection of mosquitoes. To a certain extent they are typical carriers and should be actively treated from a standpoint of malarial prophylaxis. Provocative measures to induce a relapse with a reinvasion of the blood by the parasites is discussed on page 90.



FIG. 27.—Typical malarial spleen in untreated children of the North Argentine (After Muhlens)

**Masked Malaria**—Cases have been reported under the term of masked malaria in which the symptoms have been particularly neuralgic or gastro-intestinal in character or in which various skin eruptions appeared and which showed periodicity and responded to treatment with quinine. A definite diagnosis of malaria should not of course be made in such cases unless malarial parasites can be demonstrated in the blood. During the quiescent stages of malaria it is often exceedingly difficult to make a definite diagnosis of the actual existence of the infection. There may or may not be splenomegaly.

**Malarial Cachexia**—As the result of repeated attacks of any type of malaria a condition of anaemia and physical and mental incapacity may be produced. The skin may have a dirty earthy hue particularly of the face, and the sclerae may show a yellowish tinge. The patient is often sensitive to the slightest cold and may be the victim of mental

depression with deterioration of memory or at any rate lack of concentration. Manson in earlier years particularly emphasized this condition. There may be long periods in which the temperature is normal or subnormal but slight febrile accessions may occur from time to time and at such times the blood may show parasites. The spleen is usually enlarged as may also be the liver. Twisting of the pedicle of the spleen or its rupture from even slight blows may necessitate surgical intervention. There may be anorexia and alimentary tract disturbances. A very important feature of malarial cachexia may be the occurrence of haemorrhages particularly serious being those from the retinal vessels. It is probable that hookworm infection has frequently been confused with the anaemia of malarial cachexia as in both of these conditions we may have a well marked anaemia with swelling about the ankles, palpitation of the heart and shortness of breath. Some authorities have recently called attention to splenic enlargement in hookworm disease but this is not generally found in uncomplicated cases. There may be also ascites in chronic malaria and the syndrome of Banti's disease. Urobilinuria may be an important sign in malaria where other causes for red cell destruction are excluded.



FIG 28—Malarial cachexia  
(D ad : k)

**The Sequelae of Malaria**—The anaemia and other manifestations of malarial cachexia have been described above. It is emphasized that the enlarged spleen may be a source of danger from rupture and may cause sensations of pain or tension. Hospital attendants of native patients should especially be warned of the danger of rupture of an enlarged spleen from sudden physical exertion or a blow upon the abdominal wall.

In chronic cases the spleen may come to weigh many pounds and occupy a large part of the abdomen. Other factors besides the malarial parasite may influence the enlargement. Changes in such spleens are more chronic. The capsule often thickens sometimes revealing whitish fibrous patches. Many of the spleen trabeculae may be greatly hypertrophied. In sections the spleen is moderately firm and may vary in color from reddish brown to dark brown or bluish black. Fairley (1940) has described a peculiar haemolytic hypochromic anaemia associated with post malarial splenomegaly of Banti's type.



The skin of those with chronic malaria tends to ulcerate from slight wounds and phagedenic lesions may occur. There may be various disorders of the nervous system varying from mental confusion or lack of mental concentration to melancholia. Neuritis and possibly peripheral neuritis may have origin in repeated attacks of malignant tertian malaria. However, there is considerable difference of opinion among clinicians as to whether a peripheral neuritis of malarial origin exists. Well marked neuritis has been observed in cachectic malarial patients. Nevertheless, such patients are sometimes predisposed to various infections and disturbances and vitamin deficiencies must be considered in this connection. DeLangen and Iichtenstein emphasize the occurrence of neuritis in malaria and say that it may begin during or shortly following a febrile period and is usually accompanied by pains or paraesthesias in the corresponding part of the body. Paresis may occur and may even pass on to total paralysis. Atrophy of the muscles and degeneration reactions occur as in any other form of neuritis. After reaching its height the neuritis is said to remain stationary for a time and then slowly pass off. They found the nerves of the lower extremities most often attacked. In other cases, a neuritis of the ulnar nerve or of the brachial plexus might appear separately. Occasionally the facial nerve was involved. Apparently these cases were observed in regions where beri beri was common. Optic nerve neuritis was observed in connection with malarial amblyopia. Cases with paralysis of the abducens nerve were also seen as well as of paresis of the vocal cords. They point out that polyneuritis has been observed in the course of malaria but that its etiology is doubtful as a latent beriberi may be aroused to activity by the attack of malaria.

Malarial amblyopia will be discussed under the special senses. Ulceration of the cornea is the most frequent of the ocular sequelae although even this is rare. It only occurs after many relapses. It is painful, heals slowly, and tends to recur with relapses. Iritis may accompany it. Abortions are frequent unless the malaria is adequately treated.

### SYMPTOMS IN DETAIL

**General Appearance**—In the cold stage of the benign infections the face is pinched and blue to become decidedly flushed when the hot stage sets in. In typical malarial cachexia there is an earthy color of the skin with the pigmentation more marked about the face and knuckles. In the algid forms of pernicious malaria the skin is pale, cold and clammy in a measure simulating cholera. Herpes labialis is very common in the benign infections but less so in the malignant tertian ones. Jaundice is a feature of bilious remittent fever.

**The Temperature**—Even in the cold stage the temperature is steadily rising and may have reached 105°F or higher by the time of onset of the hot stage. It remains elevated during the 4 to 6 hours of the hot stage and then falls rapidly to normal during the sweating stage. The paroxysm tends to occur usually in the forenoon or early afternoon. In 793 typical paroxysms Stott found only 37 per cent to occur before noon. Intermit

tent fever curves are characteristic of benign infections. In malignant tertian a prolonged hot stage (15 to 36 hours) is a marked feature. The onset also is more gradual and the fever tends only to remit or may remain continuous over several days but even with such a chart they are apt to be indications of slight rises every other day. In the hyperpyrexial form of cerebral perniciousness the temperature may rise to  $112^{\circ}\text{F}$  and the case resemble sunstroke. In the algid forms the axillary and rectal temperatures are usually elevated.

*The Circulatory System*—The pulse is small, rapid and of high tension in the cold stage to become full and bounding but rarely dicrotic in the hot stage. A cardiac type of perniciousness in which the right heart dilates has been referred to. Dudgeon and Clark believe death from sudden cardiac failure may be due to toxic fatty degeneration of the myocardium.

*The Alimentary Tract*—Nausea and vomiting are common manifestations of malarial paroxysms and in bilious remittent fever the vomiting is an especially distressing feature. So called choleric form and dysenteric manifestations of perniciousness of the algid type are rather rarely observed. Still more rare are cases with the clinical picture of acute haemorrhagic pancreatitis which have been reported as incident to excessive sporulation of malarial parasites in the capillaries of the pancreas.

*The Respiratory System*—There may be a slight bronchitis in ordinary types of malarial fever. In the cerebral types of perniciousness the breathing may be markedly altered—even of Cheyne Stokes character. A broncho pneumonia which shows a periodicity and responds to quinine has been described as a manifestation of pernicious malaria.

*The Skin*—Herpes labialis is a common manifestation of benign tertian and not rarely of malignant tertian infection. Urticaria may also be noted. The skin of malarial cachexia as noted is often earthy in hue. One must always keep in mind the skin eruptions due to quinine administered in treatment and of these urticaria is probably the most frequent.

*The Nervous System*—In both benign and malignant infections headache is a marked feature and is accentuated during the hot stage. There may be a condition of mental confusion in the hot stage of benign tertian and quartan but in aestivo autumnal infections particularly there may be actual delirium. Delirious and comatose states are prominent features of cerebral pernicious attacks. At times there may be an apathetic condition suggesting typhoid fever. Different types of disease of the central nervous system may be simulated as the result of focal sporulation so that we have aphasic epileptiform hemiplegic bulbar and other clinical types. Some authors have recorded cases of simple and also multiple neuritis of malarial origin. (See sequelae page 76.) Catto examined the blood of a number of cases of multiple neuritis in Jamaica but obtained negative malarial findings in every case. However neuralgic manifestations may be a feature of the latent forms. Some loss of memory is not uncommon after severe malaria.

*The Special Senses*—Malarial amblyopia occurs as a somewhat rare complication and has been attributed probably to the action of the

malarial toxin upon the optic nerve and retina. Loss of vision is usually only transient but there may be complete blindness lasting from several hours to some days or even months. In the latter case optic neuritis peripapillary oedema, blocking of the retinal and choroidal vessels by parasites and leucocytes and multiple haemorrhages in the fundus are sometimes present. In some of the cases the ophthalmoscopic findings have been reported as negative in others the disk was rosy or cherry pink in color. These fundus changes must be distinguished from and differ from those of quinine amblyopia or amaurosis. While the latter usually follows the ingestion of a large quantity of quinine as little as 12 grains (0.78 gm.) may produce temporary amblyopia in susceptible individuals. In quinine amblyopia the condition depends on the retinal anaemia from the toxic spasm of the arterioles. Extreme palor of the optic disks, a marked diminution of the retinal blood vessels in number and caliber and contraction of the field of vision have been reported. The pupils are usually dilated. The restoration of central vision which is the first to recover may be perfect or incomplete. Occasionally blindness has been permanent.

A form of dendritic keratitis due to malaria has been described especially by Kipp and by Elliot. It commences as a herpes of the cornea but without the appearance of definite vesicles. Superficial ulceration follows. Lateral offshoots develop and the affection may pass on to present the characters of a well marked dendritic ulceration. It is usually associated with photophobia and lacrimation and sometimes is ushered in with severe supraorbital neuralgia. Elliot reports that retinal haemorrhages are a comparatively frequent occurrence in malaria. They are frequently overlooked when small as they are so far forward they are difficult to see with an ophthalmoscope and as they lie in the periphery of the retina positive symptoms are generally not present. Also a routine examination of the periphery of the fundus is seldom made in malarial patients. The haemorrhages are believed to be due to blocking of the capillary vessels by parasitic cells or to changes set up by the parasites in the lining cells. Larger retinal haemorrhages in the macular area have been sometimes observed associated with cachexia of malignant malaria. Elliot states that if the cases in which blindness is due to haemorrhages occurring in the central region of the eye are excluded it may be questioned whether malaria is ever responsible for blindness but it is quite certain that quinine often is.

Usually the effect of quinine upon the ear is manifested by deafness and tinnitus.

*The Genito urinary System*—In the cold stage there is apt to be frequent urination with increased secretion. Later on the urine may be scanty. Albuminuria is rather common in aestival autumnal attacks and true nephritis has been reported in about 2 per cent of cases in some series. Sinton and Lal found albuminuria in 14 per cent of 467 cases. MacFie, Lambers and Manson Bahr (1940) lay stress on the reports of nephrosis as a complication or sequel of malaria and especially in quartan

infections Plehn attaches great importance to the examination of the urine for urobilin as showing malarial infection when parasites cannot be found. Bile in the urine is an important sign of bilious remittent fever. Orchitis has been reported as a malarial complication.

*The Liver and Spleen*.—Tenderness over the liver and jaundice occur especially in bilious remittent fever. Under the influence of a succession of attacks the hepatic congestion may gradually result in a more or less permanent enlargement. The spleen is the organ in which the infection particularly centers and its tenderness and enlargement are of special diagnostic value in malaria.

Splenomegaly is considered on page 82. Even in comatose conditions pressure on the spleen may bring about indications of pain. The liability to rupture of the friable spleen of aestivo-autumnal infections has been considered and must not be disregarded and the patient should not expose himself to injury.

*Malarial (Endemic) Index*.—Various procedures have been employed to estimate quantitatively the prevalence of malaria in a region in which it is endemic. The percentage of mosquitoes which are infected may be determined by dissection, an examination being made of the stomach wall of suspected females for oöcysts or sporocysts and of the salivary glands for sporozoites. The salivary gland index (i.e. sporozoite rate) is considered the more valuable since it indicates the complete development of the plasmodium in the mosquito. In heavily infected areas the sporozoite rate of naturally infected *Anopheles* has been generally less than 5 per cent. King found in the southern United States in *A. quadrimaculatus* a rate of 0.107 to 0.57. Boyd in Brazil in *A. albipennis* 2.8. Stephens and Christophers in India found in *A. culicifacies* a rate of 4.6 to 8.6. Paul Russell in the Philippine Islands found in *A. minimus* 0.3. In East Macedonia where the races of *Maculipennis* (*messege typicus* and *clutus*) occur together Barber and Rice who dissected about 37,000 specimens in 3 years found *clutus* 1.29 per cent infected and *messege* and *typicus* 0.07. On the other hand in Natal, Brazil, Shannon and Barber found in *A. gambiae* a heavier sporozoite index (from 2.7–10 per cent) where the rate of infection in the population was high.

However, as regards natural infection of malaria in mosquitoes many factors which influence these results must be considered. For example the season of the year at which the investigations were conducted, whether malaria was prevalent or scanty at the time, whether the insects were collected in human habitations or in stables and sheds where domestic animals were present. Thus Bentley in Bombay found that 18 per cent of *A. stephensi* were infected in August but none in the dry season. King found that while the general infection of *A. quadrimaculatus* during 12 months was 0.57 per cent in the specimens taken in negro huts it ran as high as 4.9 per cent.

The parasitic index (Ross) is the percentage of individuals who show parasites in the blood and obviously sometimes underestimates the incidence of infection in varying degree. Christophers, Sinton and Wilson

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The *parasitic index* (Ross) is the percentage of individuals who show parasites in the blood and obviously sometimes underestimates the incidence of infection in varying degree. Christophers, Sinton and Wilson

(1939) have suggested counting the number of parasites in the blood during a malarial survey for giving more accurate information of the susceptibility of a community to malaria but this method may give very unreliable data since the number of parasites in the peripheral circulation at certain times may be nil and not in accord with a severe infection of internal organs

The *splenic index* is the percentage of individuals who show enlargement of the spleen by palpation while the *endemic index* includes all who show either parasites or splenomegaly. The ease with which the splenic index can be determined in a population and over large areas in a very short space of time has made it a most valuable method of giving a rough estimate of the prevalence of malaria in the population of many countries as in the southern United States. In regions where other forms of splenomegaly do not occur in great proportion, it is particularly valuable. Many distinguished malarial epidemiologists, such as Ross, Darling, Christophers, James, Watson, Schüffner, Swellengrebel, Barber and Boyd are convinced of its great value in estimating the amount of malaria. However, Stephens and Christophers believe that it is a safe guide only in children between the ages of 2 and 10 years. So it is valuable to guard against the tendency to overestimate the value of the splenic index particularly in adults.

One difficulty with reference to the relationship of splenomegaly to uncomplicated malaria is the lack of an accurate method for determination of splenic volume in man. Christophers and Schüffner have made especially intensive studies in an effort to find a means of expressing more accurately the size of the spleen. However, by all known methods it appears that it is difficult to detect changes of the spleen size unless these are of considerable magnitude. Missiroli has expressed the opinion that the spleen may sometimes increase to twice its normal size before it is palpable.

In certain malarial surveys performed especially in India it has been suggested that a region in which demonstrable splenic enlargement is found in less than 10 per cent of the cases should be regarded as healthy, enlargement in from 10 per cent to 25 per cent indicates moderate endemicity, from 25 per cent to 50 per cent indicates high endemicity and from 50 per cent to 90 per cent or higher hyperendemicity.

While there has been considerable difference of opinion expressed from time to time about the value of the splenic index in the diagnosis of malaria, palpability of the spleen, temperature curves and cyclic manifestations should not generally be considered by the practitioner reliable for diagnosis without microscopical investigation.

Moreover, in districts in which infantile or adult leishmaniasis causing splenomegaly is present or where schistosomiasis occurs the splenic index while still of some value in the determination of the presence of malaria cannot give alone as accurate information as the examination of the blood or the fluid obtained from the spleen by puncture. However, in other localities and especially in children it may be valuable.

Barber working in the Philippines with children from 5 to 10 years of age obtained a spleen index of 12.3 and a parasitic index of 11. In making a spleen index it is best to separate children of different races, ages and environments.

Clark in an extensive study of West Indian negroes upon the value of palpation of the spleen and examination of thick blood films found in the examination of 11,000 adults a parasite rate of 23.5 per cent and a spleen rate in the same persons of only 3.5 per cent. In other words only 110 of the 2,585 adults whose blood films were positive for malarial parasites had also palpable spleens. If palpation alone had been relied on in this survey the diagnosis would have been missed in 2,475 of 2,585 positive cases. In children of whom 1,102 were examined the parasite rate was 41.0 per cent and the spleen rate was only 22.78 per cent. That is only 175 of the children whose blood films were positive for malaria had palpable spleens. If palpation alone had been employed the diagnosis would have been missed in 287 of 462 positive cases. Clark's careful investigations demonstrated that as a quantitative method for selecting adult males in need of treatment for malaria palpation of the spleen is unreliable and that its success is limited even when applied to children.

Clark and Wilson in the study of the positive blood films of the children found that 86.19 per cent showed aestival autumnal parasites, 11.23 per cent quartan, 0.49 per cent benign tertian, 1.18 per cent mixed infections and 11.2 per cent crescents. The percentages for adults were approximately the same except that 8.87 per cent were crescent carriers. In emphasizing that the spleen index is of but little value in estimating the prevalence of malaria in Haiti they suggest it may be accounted for in the high racial tolerance or the high incidence of aestival autumnal infection as compared with quartan and benign tertian.

However opinions differ regarding the differences in the size of the average spleen resulting from infections with *P. vivax malariae* and *falciparum*. Covell and Bailey (1927), Sweet (1933) in India and Boyd (1930) in the United States imply that the greatest degree of splenomegaly occurs in infections with *P. vivax* and that in malignant tertian infections there is a higher proportion of smaller spleens. However Barber and Rice (1937) in their survey of malaria in Egypt where the subject was carefully studied found that among persons infected with *P. vivax* a much higher percentage had negative spleens than was usually the case in infections with the other species of *Plasmodium*. In these studies they made examinations of 1,962 individuals in all of whom both blood and spleen were studied. The percentage of spleen enlargements was not only smaller in the *vivax* infections but this was true of every grade of splenic enlargement and especially so of those of higher degree.

Recently Genevray (1938) found in China that the high spleen rate was only 2.4 per cent whereas the parasite rate was 90 per cent and the gamete rate over 80 per cent. MacDonald who has made wide studies of malarial infection in children both in West Africa and in India believes



that only some 50 per cent of infections with malaria are of sufficient severity to produce clinical enlargement of the spleen. As a result of his final studies in Assam he concludes that the parasite rate reaches its height in heavily endemic areas in the first two years of life after which it decreases and that the spleen rate reaches its height at from 3 to 6 years and thereafter declines. He finds that a large spleen is generally associated with a moderate parasitic infection whereas small spleens are associated with either a very small or very high parasite count.

These clinical observations are of interest in connection with the experimental work of Coggeshall (1937) who made a study of splenomegaly in experimental monkey malaria and has designed an apparatus and a technique which makes it possible to measure accurately changes of spleen volume in living monkeys. He followed these changes in a total of 21 animals and found an important response in the size of the spleen after inoculation which may even precede the presence of parasites in the peripheral blood. The least degree of splenomegaly was associated with infections caused by the most pathogenic parasite employed *P. knowlesi*. These infections all terminated fatally. A slightly larger spleen was found when the course of the disease was extended by decreasing the size of the dose either in actual numbers of parasites or by giving the inoculation intramuscularly instead of intravenously. In other instances the spleen ceased to enlarge and frequently decreased in size near the fatal termination of the disease. Monkeys infected with the least virulent parasite *P. vivax* showed a beginning splenomegaly at the time parasites were visible in the peripheral blood. At the peak of the acute stage of the disease the spleen attained its maximum size sometimes being approximately 4 times its original size. Superinfections produced an accelerated rate of spleen enlargement as compared with primary attacks but with equal rapidity the spleen returned to the size noted before superinfection.

It seems probable that in man the splenic reaction to malarial infection varies not only at different ages but in different individuals and races as well as according to the species of infecting parasite and the severity of the infection.

**Splenomegaly**—There is a very great difference in the prevalence of marked splenomegaly on the West Coast of Africa as compared with that which exists in parts of the Amazon basin in Brazil. Malaria widely prevails in both regions but on the West Coast of Africa advanced splenomegaly is not a striking feature whereas in the Rio Branco regions it is and large spleens to or below the level of the umbilicus are common.

Ziemann emphasized the fact that marked splenomegaly is comparatively rare in association with malaria throughout the Cameroons.

Lambert and Olivera in seven cases of lethal malaria found only one spleen weighing as much as 820 grams. In 3 of the cases the spleen weighed only 150 grams or less while in a number of other cases of subsidiary malaria where death occurred from other causes the spleen was either not distinctly over weight or was even somewhat less than the normal weight of 150 to 170 grams.

Clark found in Central America (the Caribbean area) that while extreme cases of splenic enlargement 1000 grams or more in weight were not infrequent in the Latin American labor class they were very rarely encountered in the negro even in those who had lived for a number of years on the mainland under the same environment as the Latin Americans.

In other parts of Africa splenomegaly very frequently follows infection with *Schistosoma* as emphasized by Ferguson and Day and by Rich

ards and more recently by Bonnin Schweizer Coleman Stiven and Gorges

In Amazonia Davis (1934) has recognized *S. mansoni* infection in 1694 sections of livers obtained with the viscerotome from patients dying in northern Brazil. It is important definitely to determine whether all the splenomegaly of Amazonia is a manifestation of malarial infection or whether some of it does not represent a form of splenic anaemia in which some other cause is at least a contributing factor.

Dee and Tribendi (1939) in discussing the commoner types of splenomegaly in India mention that due to malaria leishmaniasis and a third type which resembles clinically Banti's syndrome in which no infective agent was recognized. They term this type Bengal splenomegaly. It apparently is the same condition which Councilman and Lambert and Shattuck and the writer observed especially in the Amazon region and elsewhere in the tropics in earlier years and which has been termed tropical splenomegaly.

Rousellot and Thompson (1939) have tried to produce experimentally Banti's syndrome in monkeys by splenic vein constriction but their results were unsuccessful as either complete venous occlusion developed with splenic atrophy or an adequate collateral promptly formed with no alteration in the size of the spleen. However they finally found that the injection of fine siliceous particles directly into the splenic vein will produce a progressive cirrhosis of the liver. Secondary to this a state of splenic vein hypertension was produced with a concomitant congested splenomegaly.

**Therapeutically Induced Malaria for General Paralysis**—Since 1917 the use of infection with malaria in the treatment of general paralysis first suggested by Wagner Jauregg has been reported from various parts of the world. It has also been used in the treatment of other forms of neurosyphilis. Patients have been infected either by inoculation of blood of persons harboring the benign tertian parasite or by subjecting them to the bites of infected anophelines. More rarely the subcutaneous inoculation of extracts of the salivary glands of insects containing sporozoites has been employed.

The method in which the bites of infected mosquitoes is used is to be preferred as it avoids the danger of the direct inoculation of the syphilitic virus or any other pathogenic organism from another human host and also the accidental inoculation of malignant tertian parasites can more readily be excluded.

The disadvantages of this method of infection induced by a mosquito bite is that it is less susceptible to quinine treatment and more likely to relapse and the procedure is more complicated as a group of infected mosquitoes must be kept on hand.

Great danger may result from the injection of malignant tertian blood on account of the frequent virulence of this parasite. It has been suggested that by incubating the anophelines which have fed on malarial patients at about 22 C. the malignant tertian parasite requiring a

higher temperature than the benign tertian one has its development in the mosquito inhibited. After the mosquitoes have become infective they are kept in the ice box at  $5^{\circ}\text{C}$ . Under such conditions they usually will live and remain infective 10 weeks. This attests the known persistence of malaria in the hibernating mosquito. A remarkable fact is that only a small proportion of gametocyte harbouring patients infect mosquitoes which feed on them. This may be due to variations in the abundance of gametocytes but there is a question of difference in infecting quality of the blood of different patients.

Gametocytes first appear about a week after the onset of the malaria and the earliest sexual parasites do not seem to infect mosquitoes—this being brought about by gametocytes present in the blood 10 days or later after the onset.

For successful infection of the mosquito repeated blood feedings may be required. Fruits or other feedings may change the optimum reaction for infection of the mosquito's body fluids. As a result of their studies James and Shute feel that it is only with those anophelines whose environment approximates natural conditions that infection of man is probable.

The benign tertian parasite has been used in most cases. Quartan malaria has been given particularly to patients who are resistant to benign tertian particularly to negroes or to those who recover prematurely. Plehn considers that where the production of benign tertian malaria has failed to benefit a patient we are justified in next treating the patient with malignant tertian parasites. Nevertheless the mortality has been high in some series of Caucasians inoculated with this species. Both *P. falciparum* and *P. knowlesi* have been used successfully in negroes. Several investigators have also recently inoculated *P. ovale*.

In the United States and England both the methods of direct inoculation of blood and of transmission by infected mosquitoes have been employed. In the direct inoculation 5 cc. of blood preferably obtained at the height of the paroxysm may be injected subcutaneously or sometimes more effectively intravenously. Good results have also been obtained from the injection of only 1 or 2 cc. of blood. The blood need not be matched as to group. If an infection is not obtained larger amounts of matched blood (up to 100 cc.) may be given intravenously. By such amounts there is greater assurance of infection and the incubation period is shorter. It is said that there is no real danger of superinfecting a patient who has syphilis with a different strain of treponemata by giving blood from a case of late neurosyphilis.

After an incubation period of usually 3 days to a week or 10 days with direct inoculation the temperature becomes irregularly elevated for 2 or 3 days then quotidian paroxysms of fever begin and as a rule these soon become tertian if the infection is not quickly interrupted. If the patient's condition permits the infection is allowed to continue until from 8 to 12 paroxysms have occurred. Quinine may then be given. 5 grains three times a day will usually stop the fever after 2 days but the drug should be continued for at least 2 weeks as a precaution against relapse. Patients must be watched with great care and quinine must be administered at once if their condition becomes serious particularly if the blood pressure falls below 70 mm. if tachycardia becomes marked if the haemoglobin falls materially below 50 per cent if the parasites become excessively numerous or if renal insufficiency or marked jaundice develops. The urine must be watched with special care. In severe infections it is sometimes possible by giving 1 or 2 doses of 5 grains of quinine to change the incidence of the paroxysms from quotidian to tertian or to interrupt the fever for 2 or 3 days without terminating the attack.

Re inoculation is usually possible during the next 2 or 3 months if the attack did not terminate spontaneously but after a longer interval the patients sometimes become resistant to re inoculation with the same species and remain so for as long as several years.

The inoculated disease (man to man) usually differs from the natural infection by mosquitoes in that the fever is more irregular the paroxysms are less clear cut and the late recrudescences are never observed. Subjects for inoculation should be selected with care.

Contraindications to infection are myocardial or renal disease anaemia lowered liver function senility and advanced emaciation. Liver function may be examined by the bromsulphalein dye test. However a normal result does not necessarily exclude liver disease. Before treating cases with induced malaria, it is advisable to obtain a control blood count, and liver function dye test and serum bilirubin (van den Bergh). If these are normal and no contraindications for inducing malaria are present the treatment may be given with apparent safety. After the malaria has been induced the case should be followed by a blood count and serum bilirubin test after each chill and a bromsulphalein dye test once each week. Without these tests one could not tell whether the malaria was producing anaemia bilirubinaemia or liver damage or whether these had been present before beginning treatment. See Fig. 29.

Treatment should be interrupted at any time the patient shows algid manifestations anaemia to the degree of three million red cells per cubic millimeter cardiovascular collapse evidence of liver damage as determined by the bromsulphalein dye test and marked increase in serum bilirubin as determined by the van den Bergh test. Symptoms indicating gastro intestinal tract disturbance or evidence of kidney damage should warrant termination of treatment.

Certain differences have been reported regarding the incubation period with the different species. Following direct inoculation Boyd and Kitchen (1937) found that the minimal period between the injection and the detection of *P. falciparum* parasites in the blood was 6 days or 2 days less than in the case of *P. vivax* infections. The parasites were most commonly first found in *falciparum* infections from the 11th to the 13th day the longest period being 25 days. In mosquito transmission Boyd found the incubation period with *P. falciparum* varied between 8-15 days averaging 11 days. With *P. vivax* the incubation period was never less than 9 days and infrequently over 20 days. In a few instances it varied from 85-97 days. The incubation period was found to be directly proportional to the dose of the sporozoites. In the case of *P. malariae* the incubation period has been found in at least some instances to be longer 27-42 days. The attacks were more severe than observed in *P. vivax* infection and Boyd regards the disease in general as more severe than that produced by *P. vivax*. In two negroes successfully inoculated the acute illness observed was of relatively short duration as compared with that seen in whites. Gametocyte production was not observed until several months after the onset of the disease and was never extensive. Hence Boyd concluded that it is not well adapted for general therapeutical use because the supply of infected mosquitoes cannot be frequently renewed.

One factor that has developed from the study of therapeutically induced malaria is the racial immunity that may exist in negroes especially to *P. vivax* infection. This immunity or tolerance is relative and not absolute as has been shown by an occasional successful infection of a negro. Thus Foyd who inoculated 14 negroes with *P. vivax*

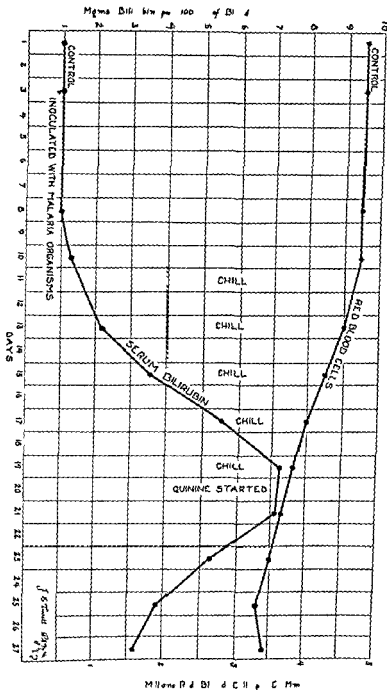


FIG 29—The rise in the serum bilirubin in a case of malaria as studied by the quantitative van den Bergh reaction. It will be noted that the red blood cells decrease in number as the serum bilirubin increases.

had only 5 takes and in only 3 of these was there evidence of any clinical reaction Milam and Kusch (1938) who inoculated 29 white patients with *P. knowlesi* found them all susceptible to infection. However 6 negroes reacted very mildly or not at all.

A number of investigators believe that certain Caucasians also show greater resistance to infection to malaria than others and in some of the series of inoculations this seems evident. Thus James (1931) considers that in their degree of natural susceptibility to the pathogenic effects of primary infection with *P. vivax* individuals may be grouped roughly in 3 classes (1) Those who are very susceptible to malarial infection (2) those who are relatively quite refractory and (3) intermediate between these 2 extremes the vast majority of people who may be described as normally susceptible. His later studies (1932) suggest that similar grades of susceptibility may exist against infections with *P. falciparum*.

Sinton Hutton and Shute (1939) also found in the treatment of a series of cases with *P. ovale* that similar grades of relative susceptibility for this parasite existed among 68 patients who were with a few exceptions Europeans who had never been exposed previously to infection with *P. ovale*. In one of James' series approximately 18 per cent of the cases inoculated failed to develop an attack of malaria within the usual incubation period. He believes that the failure encountered could not be explained as being due to failure of the mosquito to inject a sufficient dose of sporozoites but rather to a condition of refractoriness in the patient. However Boyd who attempted infection with *P. vivax* by mosquitoes in 103 patients found 87 positive results and 16 failures. Of these 12 later were reinoculated and infections were obtained in all. Boyd believes that failure to become infected in these experiments was dependent upon the deficiencies in the parasite rather than in the human subject.

**Value of Treatment**—Some difference of opinion has been expressed as to the value of malarial therapy in the treatment of general paresis. However Solomon (1937) in summarizing such work states that either by febrile treatment or tryparsamide it has been possible to arrest the active process of the spirochaete in at least 70 per cent of the cases and that approximately in 30 to 40 per cent there has been sufficient clinical improvement to return the patient to his former place in society. He believes that by the use of fever results are obtained more quickly but that the combination of fever and tryparsamide treatment affords the best chance of success and the continued use of tryparsamide affords a greater guarantee against relapse. It is now generally believed that the favorable results are due to hyperpyrexia in itself and not to any other action produced by the malarial parasites. It has also been suggested that the improvement in the treatment of general paresis may be due solely to the hyperpyrexia but whether it is due also to the stimulation and production of phagocytes by the reticulo endothelial cells is a matter of controversy.

Boyd and his associates (1938) in their report of 190 cases of cerebrospinal lues in which malaria therapy was supplemented by chemotherapy found that 55 per cent of the cases receiving malarial therapy as contrasted with 30 per cent of those receiving chemotherapy alone showed varying degrees of improvement to complete remission while 45 per cent of the former and 70 per cent of the latter were either unimproved or had died. The results from naturally or artificially induced malarial attacks did not

appear to be significantly different. The present status of the patients in the 2 series at the time of the report was as follows:

	Malaria therapy plus chemo therapy per cent	Chemotherapy alone per cent
Remission	31.6	20
Improved	23.2	10
Unimproved	19.5	25
Dead	25.8	45

It was estimated that only deaths occurring within one month of the cessation of the attack of induced malaria could fairly be laid to treatment. Of the 49 reported dead, only 13 died within that period, which would give a mortality rate due to treatment of 6.9 per cent. They conclude that malaria therapy combined with chemotherapy gives very much better results in the treatment of neurosyphilis than chemotherapy alone. They believe it advisable in the treatment that the patient should be subjected to a minimum of 21 paroxysms which attain a height of 104 F or more. In their hands, a single infection with the quartan parasite appeared most frequently to produce this result.

**Dangers of Transmission by Blood**—Transfusion of whole blood has become very common in clinical therapeutics, but little attention has in many instances been paid to the transmission of other diseases by the transfusion except syphilis. It therefore is not strange that malaria has in a number of instances been accidentally inoculated. Wright (1938) has collected 29 cases from the literature in which the disease was transmitted by transfusion. Gordon (1941) has reported 6 additional ones, the last from the administration of stored blood. Gardner and Dexter (1938), in reporting a case of quartan malaria contracted through transfusion, record the fact that *P. malariae* can remain latent in the internal organs of the body for long periods.

Naviero has reported a case of quartan malaria in a 6 weeks old infant transmitted by the intramuscular injection of blood from the father who had had malaria 37 years previously in Italy. The parasites were still demonstrable in the father's blood. McCulloch described the case of a woman in Toronto who contracted quartan malaria following transfusion from a donor who had had malaria in Pumania 25 years before and had no symptoms of the disease in the interim. Wright described an infant in whom malaria developed 4 weeks after transfusion from the father who had been free from symptoms for 33 years.

Wang and Lee (1936) have also reported that 59 cases of benign tertian fever and 6 cases of relapsing fever have followed blood transfusions in Peking Union Medical College in the past 10 years. These cases emphasize the care that should be taken in the examination of the donor's blood for malarial parasites and other microorganisms before use in transfusion. They also point out the great danger of employing blood for transfusion from a donor who has resided in a country where malaria is epidemic.

**Infection in Drug Addicts**—A number of infections of man with malaria have been reported among drug addicts who have used the same unsterilized syringe, one of them having been previously infected with malaria. Appelbaum and Gelfand observed 39 cases of malaria among

drug addicts in Bellevue Hospital New York City where the infection was said to have occurred from the use of diacetyl morphine a small amount of blood (from an individual suffering with malaria) having been first sucked up into the syringe in order to be sure the needle had entered the vein and the other patients subsequently inoculated with a portion of the contents Biggam has also reported such infections in Cairo due to intravenous injections of heroin among drug addicts All his cases clinically were of the malarial-dysenteric type

Helpert (1934) Jolliffe (1940) and Most (1940) have also emphasized the importance of this method of infection in drug addicts and the fatalities that may result from such infection Most during two years observed over 100 heroin addicts suffering from *fulciparum* malaria contracted as a result of the common use of a hypodermic syringe for intravenous administration of the drug Clinically and pathologically the disease manifests all the characteristics of *fulciparum* malaria as it occurs in the tropics including various cerebral gastro-intestinal and haemoglobinuric syndromes Among the cases with cerebral involvement stupor and coma were the special symptoms observed in 10 of the fatal cases Most points out that this disease has become endemic in the New York metropolitan area

### DIAGNOSIS

In the diagnosis of malaria the special points to consider are (1) Presence of malarial parasites (2) Periodicity (3) Splenic enlargement (4) Response to quinine therapy (5) The presence of melaniferous leucocytes (6) A high large mononuclear percentage when leucopenia is present

In the examination for parasites it is of importance to consider the species of parasite present and the stage of development and the presence of the sexual forms In an intensive investigation Bass found that 55.09 per cent of those showing parasites in the blood gave a clinical history of malaria while 44.91 per cent of those with parasites in the blood revealed no clinical manifestations of malaria Blood platelets have been frequently mistaken for malarial parasites in stained blood and vacuoles for young parasites in fresh blood Quartan and tertian periodicity is only found in malaria but quotidian periodicity is a feature of a host of diseases

**Differential Diagnosis**—There are very few tropical diseases which have not been mistaken for malaria and in many instances the affection has been considered to be of malarial etiology before the discovery of the real cause

As regards tropical diseases—kala azar Malta fever live abscess filariasis trypanosomiasis leprosy relapsing fever and yellow fever are to be thought of in differential diagnosis Liver abscess has been frequently confused with malaria

Of the cosmopolitan diseases typhoid fever septic conditions including malignant endocarditis tuberculosis influenza pyelitis and even syphilis are to be considered in a diagnosis of malaria

As was noted under the discussion of the pernicious manifestations of malaria many diseases may be simulated by the sporulation of the malarial



parasite in certain organs or areas of organs. One should always keep in mind the possibility that pain in the appendix region or in the gall bladder may be connected with malaria if in the tropics. A polynuclear increase negatives malaria and may suggest appendicitis or cholecystitis in such instances.



FIG. 30.—A lust of blood platelets and two platelets lying upon a red cell and a malarial parasite. ( $\times 1000$ ) (Todd)

Malarial cachexia must not be confused with hookworm disease or other secondary anaemias due to intestinal parasite.

*Provocative measures* to induce a relapse with a reinvasion of the blood by malarial parasites are occasionally employed for diagnostic purposes.

Among these may be mentioned fatigue, refrigeration, exposure to sunlight, administration of small doses of quinine for 10 to 14 days or of berberine sulphate, intravenous injections of typhoid vaccine and subcutaneous injections of ergotine, strychnine, adrenalin and epinephrin. Injections of epinephrin have been generally regarded as the most effective procedure. After the subcutaneous injection of 1 cc. of a 1 to 1000 solution of epinephrin the parasites may appear in the blood as early as 20 minutes after the injection and reach their height in about 1 to 2 hours. The injection of this drug usually causes a marked increase in the number of erythrocytes, platelets and leucocytes, probably as a result of the vasoconstriction of such organs as the spleen, marrow and other haematopoietic centers.

Levi Simpson and Kadness (1936) have performed experiments on guinea pigs with a closely related drug, ephedrine, and demonstrated (by splenectomy) that the spleen is not essential for a rise in red cells, leucocytes and platelets, and that the drug causes extrusion of these cells into the circulation from storage and haematopoietic centers including the bone marrow.

Others have employed 2 cc. of adrenalin hydrochloride dissolved in 200 cc. of normal saline solution. This may result in reduction of the size of the spleen as well as the appearance of parasites in the blood.

Videla (1934) has reported successful results in the demonstration of parasites in 4 of 6 cases in which the usual methods had failed by daily intravenous injections of 10 cc. of 10 per cent calcium chloride solution for 2 or 3 days.

During the winter parasites tend to disappear from the circulation regardless of treatment. Obviously the accurate diagnosis of malaria can only be made by laboratory examinations.

**The Blood Examination**—This is of prime value in the recognition of malaria, and one should examine both fresh blood preparations and stained films with the  $\frac{1}{12}$  oil immersion lens. A diagnosis of malaria

should not be made unless malarial parasites are definitely detected. To the experienced observer the diagnosis in many cases is most conclusively made from the examination of a fresh preparation. In such a preparation flagellated forms may be observed but they develop only from 15 to 20 minutes after taking of the blood. Of great differential value is the swollen pale infected red cell in *P. vivax* infection as contrasted with the normal sized or slightly smaller red cell of *P. malariae* infection and the often distorted shrunken red cell of *P. falciparum* infection. The motile amoeboid forms as well as the larger pigmented parasites are quite distinctive. At an autopsy both fresh and stained smears may be made from the spleen or bone marrow. The parasites usually preserve their form longer in the bone marrow before disintegrating. Films fixed in absolute methyl alcohol should be stained by Giemsa's method and examined with the  $\frac{1}{12}$  oil immersion lens. The body of the parasite is stained blue and the nuclei ruby red. The evenly spread stained film sometimes gives more accurate information as to species and stage of cycle than any other method (see Plate I).

However if the parasites are scanty some advisers recommend the thick film method. Nevertheless the parasites may be distorted in such preparations and it is sometimes difficult to make a diagnosis from them.

In the thick film method a drop of blood may be smeared with a pin over about  $1\frac{1}{2}$  square inches of a slide and allowed to dry for 20-30 minutes in the incubator. In order to take the blood the slide is then immersed in a Petri dish containing a solution of 1 per cent formalin and 1 per cent glacial acetic acid. This process must be carefully performed in order not to detach the film. The slide is then well washed in distilled water and stained with dilute Giemsa (one drop of the stain to 1 ccm. of water) for 20 to 30 minutes, washed in water and allowed to dry without heating or blotting. Field (1940) reported excellent results for thick films from the use of the following stain:

Brilliant cresyl blue	1.0 gram
Disodium hydrogen phosphate (anhydrous)	1.0 gram
Potassium dihydrogen phosphate (anhydrous)	1.25 gram
Distilled water	100 cc

The smear may be lightly fixed in a flame, stained for one second and differentiated in clean tap water for 5 minutes.

Many observers prefer the concentration method of Bass and Johns.

*Concentration Method of Bass and Johns*—This procedure is more tedious than the preparation of thick films but the parasitized red cells are effectively concentrated and the red cells and parasites are perfectly preserved and stain as well as in ordinary thin films. Blood (5 or 10 cc) is mixed with a minimal quantity of oxalate or sodium citrate solution and centrifugalized at 2500 r.p.m. The upper layers of red cells which include practically all those containing parasites are pipetted off into a smaller tube and again centrifugalized. This is repeated once or twice more if necessary and thin films from the upper layers of the final sediment are made and stained in the usual way (Wright and Giemsa). Wilcox (1942) has published a most excellent Manual for the Microscopic Diagnosis of Malaria in Man which should be consulted for details regarding the most satisfactory technical procedures.

Blood should be examined several times a day throughout a cycle as the trophozoites of *P. falciparum* may otherwise be missed.

Crescents when found indicate a malignant tertian infection, but in some cases there may be also an additional infection with *P. vivax*. The young malignant tertian forms can often best be detected in stained films since in a fresh preparation occasionally a blood platelet lying upon a red blood corpuscle may closely resemble the young ring form. It is advisable to ascertain whether the patient has been taking quinine since if he has done so within a week the chances of finding parasites, with the exception of crescents, may be small, though occasionally pigmented leucocytes may be seen. Quinine may not only cause the parasites to disappear but may affect their staining and cause degenerative changes and such altered parasites are difficult to detect and diagnose.

Blood obtained by puncture of the spleen, a somewhat dangerous procedure or of the sternal bone marrow will sometimes show parasites in latent cases in which they cannot be found in the peripheral blood. See also Provocative Measures (above).

Schüffner's dots may frequently be recognized with Leishman's or Giemsa's stain but for the best results special details are necessary. Whitty (1937) recommends particularly Leishman's stain, employed as follows:

4 drops of the stain are placed upon the slide and allowed to remain for 30 seconds they add 12 drops of distilled water brought to a pH of 7.2 with lithium carbonate and roll the slide to effect mixture allow to stain for half an hour and flush off with the buffer solution allow to dry in the air. Giemsa (1935) also recommends a buffer solution of pH 7 with this stain.

MALARIAL PARASITES IN UNSTAINED SPECIMEN (FRESH BLOOD)

	<i>P. vivax</i> (benign tertian)	<i>P. malariae</i> (quartan)	<i>P. falciparum</i> (malignant tertian) (ae tivo-autumnal)
Character of young trophozoite	Indistinct amoeboid outline Hyaline Occupies $\frac{1}{4}$ to $\frac{1}{2}$ of a rbc	More dense and less amoeboid than <i>P. vivax</i> Frosted glass appearance of disc like parasite Size same as <i>P. vivax</i>	Small crater like dots $\frac{1}{2}$ the diam. of rbc Common to find 2 or more parasites in 1 cell
Character of growing trophozoite	Highly amoeboid (vivacious) Fine yellowish brown pigment granules	Very sluggish movement Forms more compact in outline Pigment in coarse black grains or chunks which may be in sluggish motion	Older trophozoites rarely seen in peripheral blood Fine black pigment scattered evenly in cytoplasm
Character of mature schizont	Amoeboid outline but no movement Yellow brown pigment which is motile early in schizogony	Oval in shape Coarse black pigment masses moving sluggishly in early schizogony	Not seen in peripheral blood except in moribund cases Scanty chunks of fine black pigment

## STAINED SPECIMEN

	P	P m l	P f l p m
Ch t f f et d cell	L rg d p l th o m l d ell	Ab t mal so a d l (C R m y b t t d)	Us lly n r m l L t da m di tort C H a w th l d p t a hru k d b sy
St ppling of n f t d d ll	S b ff a d t h t r t wh f d F rst pp wh t ph ost b lmost m t d B st se n h t t g	N (Z m up ph g b ght out ly by pe l t n gm th od)	B phul t pph g m y l t the d se St ph a d Chri to ph or M d ta se n lly (See t t) S lly m y lso h w b aophic t ppl g
Y r t ph t ( lly n g)	S g t r g f o m p t h d m t f d ll S gl cul N pg m t A el f m S b ff d t lly b t O e al d bl n f t f d ll	S g t r g lightly m ll d d th n P Larg d p ed hrom t n d t Cy t pl m d p bl F bl k p g m t g lly D ubl f t f ed ll	Sm ll t of th 3 p h t l g d m f b Cyt pl m th d ha tk lght bl m t h m t N pg m t B ell y f m A l o m g u l f m m m M y f d p t s p at th m d ll
G w i g t pho t ( l d n g)	I gul m b d t h with f t d f ytopl m Oft m v u l F y ll w b w pg m t grat S b ff er d t m nally	O l b d nbb d m t f m C mp t th gl v l soo d pp Bl k pg m t m d r s g m b d t b t pen ph y Th m t p z m i d p	R g j d m of d ll Oft l m t d t gl r g D t f blak pg m t ty ly d tnb ted th r g
Sch out	I d d red ll pal l g d Sh w Sch ff g l C t v ry lrr gul P y ll w bow pg m t grass Tw t to ro d d h m t m	N ly fill th l t d ell R ded d ply pg m t d d f t ly th d omp t p st w th d pbl yto plasma d d t s h ma t m	O ly se penph al bl d m n b d a O p g t s f ed ll s to d t f h m t n
M rocyt (sporu l t gach t) R it	Almost fill d t d d d ll lrr gul gr pe l w l t Y N h b w pg m t m d t lly S b ff d t m y b p s e t	N ly fill no m l ed d ll Typ l d y form w h m t g d m y m m t lly ou d t l d k pg m t m	N t se penph al bl d x e p t m n b d se P N j a t h f d ll P g m t m t r i d k brow
N ub f m sot	t 4 U lly 4 t d	6 t U lly 9	5 t 3 t l lly 4 t 6 (L p t 3 l t a)

## STAINED SPECIMEN—(Continued)

	<i>P. ax</i>	<i>P. m. la</i>	<i>P. fal. po. m.</i>
M. gam. tocyt (m. l.)	Spherical at 12m. Cytoplasm pale greyish green. h. blu. Ch. m. t. n. bu. d. nt. d. fuse. y. lep. nk. nt. lor. na. b. nd. Sc. tter. d. y. l. f. wh. b. wn. pgm. t.	Similar to <i>P. r.</i> but smaller. C. ntr. l. hu. k. of bl. k. pigment. Th. m. st. pigm. ted. of. ll. m. fo. l. p. ras. t.	S. h. rc. sto. t. sausa. g. sh. p. d. Pal. bl. cyto. pl. m. Ch. mat. n. p. le. ps. k. d. ff. s. ab. ndant. c. t. al. Pgm. nt. scat. t. d. o. e. l. s. th. nu. cleu. A. lttle. red. c. ll. cytopl. m. f. t. s. n. eo. cavity. f. enc. t.
M. rog. m. t. cyt (f. m. l.)	Spherical fill. d. st. d. d. d. c. ll. Cyt. plas. m. d. p. blue. Chr. nr. t. n. e. m. pact. de. p. e. d. ec. c. ntr. w. th. hal. r. und. it. Pgm. nt. ab. nd. t. r. th. r. rs.	Sm. l. r. to <i>P. v. so.</i> but sm. ll. Abundant brown. h. bl. k. pgm. t.	M. e. p. on. t. e. d. l. g. th. n. m. l. d. l. s. m. re. m. p. c. t. a. d. t. s. m. e. nt. s. ly. d. P. g. m. t. l.umped. n. c. gr. t. n. c. of. r.
G. alch. r. ter. f. bl. od. films	All ph. s. of sch. g. ny. p. s. t. w. th. w. d. an. ty. of am. o. b. e. d. fo. m. Mult. pl. n. f. t. n. of. d. ll. n. o. t. r. e. G. m. t. y. ly. Sch. ll. n. e. r. s. dots.	All ph. ses. f. ch. g. y. Gam. tocyt. appe. l. t. Spor. ul. t. ng. f. m. a. n. ly. se. n. at. ny. p. ro. d. f. cyc. l. M. l. t. ple. n. t. n. fr. d. r. ll. s. r. N. gr. n. ul. t. n. of. d. c. ll. y. t. pla. m.	Rings. nd. esc. n. t. a. ly. fo. m. n. in. p. r. p. h. e. f. blood. Multi. pl. n. f. t. on. f. red. fls. com. m. n. P. s. t. u. ally. m. b. n. d. nt. than. n. the. oth. r. s. f. t. na.

CAUTION—Whi. th. d. ff. nt. l. p. ts. tabulated. d. p. nd. bl. s. ar. ul. d. agn. s. sh. uld. v. be. m. d. w. th. ut. tudy. g. a. num. b. l. d. v. d. u. al. p. t. s. It. s. o. l. t. n. n. s. y. r. to. ob. u. e. sh. p. t. th. ough. an. tr. cy. l. Th. sp. s. n. t. b. d. f. f. e. r. e. nt. t. d. by. m. e. n. f. th. ly. ng. f. r. m. l. n. (s. t. t.) Th. m. t. e. r. i. o. u. s. m. s. t. k. to. v. e. l. k. P. fal. c. p. um. th. r. n. a. gl. or. m. x. d. n. f. e. ct. n. Th. r. i. g. l. m. s. e. o. f. t. e. n. d. t. e. ct. bl. n. th. p. e. r. i. ph. e. r. l. bl. d. ly. d. u. r. i. ng. a. m. ll. p. r. t. n. f. th. cyc. l.

**Cultivation** on artificial media has not proved to be a practical diagnostic procedure.

The *blood* shows a haemolytic type of anaemia which may be extreme. The red cell count may be reduced by a half million or even a million during a single paroxysm. During the paroxysm there is usually a leucocytosis. In the intervals there is a leukopenia and an increase in monocytes often to 75 or 20 per cent. This may be absent in native races long exposed to endemic malaria. The presence of pigmented phagocytes in blood films is diagnostic of malaria. Such melaniferous leucocytes are of rare occurrence and in blood which contains them parasites can usually be found in thick films.

**Reticulocyte Crisis**—If quinine is administered to a patient with malarial anaemia there is frequently a rise in the percentage of reticulocytes which reaches a maximum within 4-7 days. This is regarded by some as specific for malaria and not occurring in other types of anaemia and a sustained submaximal reticulocytosis is thought to be characteristic of an actively persisting malarial infection.

## CLINICAL DIFFERENCES

	<i>P. v.</i> (benign tert.)	<i>P. mal.</i> (quart.)	<i>P. f. l. p. m.</i> (malignant tert.)
Onset of	Usually sudden	Usually sudden	Usually sudden. Chills may be but not as with normal malarial fever.
Duration of fever	6 to 8 h	4 to 6 h	2 to 36 h or more
Duration of first attack	1 to 4 weeks	1 to 6 months	1 to 2 weeks
Typical temperature curve. First attack	Intermittent with fever 3 days then quiescence. Relapses usually 1 to 2 weeks after onset.	Usually quartan with fever 1 to 2 days then quiescence. Relapses usually 1 to 2 weeks after onset.	Night temperature at onset. Quiescence 1 to 2 days then fever 1 to 2 days.
Elimination of parasites	Usually first few days	Usually first few days	Often months or years
Clinical type	Attack of chill followed by sweating	Attack of chill followed by sweating. Quiescent periods.	Clinical type attacks of quiescence followed by fever with sweating. The fever is usually followed by a profuse type of sweating.
Rispe	May last 2 to 6 weeks and 6 to 8 months. Relapses usually 1 to 2 weeks after onset. More resistant to quinine than <i>P. f. l. p. m.</i>	Thermoregulation is first. Relapses usually 1 to 2 weeks after onset. Relapses usually 1 to 2 weeks after onset.	Relapses usually 1 to 2 weeks after onset. Relapses usually 1 to 2 weeks after onset.
Sequel	Asymptomatic	Asymptomatic. Nephritis and anemia.	Asymptomatic. Vena cava thrombosis. Relapses usually 1 to 2 weeks after onset.

Recent investigations have shown much variation in the number of reticulocytes found in health at different hours of the day as well as in disease. As they are young red cells they are usually numerous only if active blood regeneration occurs. Whitby and Britton (1937) however in accord with the usual opinion give the normal number in the blood as less than 1 per cent. However Langendorff and Reusner (1936) believe that the normal reticulocyte counts of 0.5 to 1 per cent are much too low and that they may go at times to even 11 to 17 per cent. Although these results are very unusual

this question must be more carefully studied at different hours of the day and seasons of the year as some observers have found an increase in the reticulocyte percentage during the spring (Grunke and Diesing 1936)

An abrupt rise of reticulocytes and a maximum of 5-40 per cent or even more (a reticulocyte crisis) often occurs within a few days after the institution of effective treatment in severe cases of pernicious anaemia and hypochromic anaemia. Reticulocytes also are especially numerous in *familial haemolytic jaundice* (frequently 20 per cent rarely up to 50 per cent). Fairley found after the administration of quinine or atabrin in a series of 16 cases of malaria the average maximal reticulocyte count was 10 per cent.

**Leucocytes**—Large mononuclears and transitionals containing phagocytosed pigment (melaniferous leucocytes) are characteristic of malaria—the pigment however must be in the leucocyte and not free. There is a leucocytosis during the malarial paroxysm with a leukopenia and increase in the large mononuclears (monocytes) during the apyrexial period often to 15 per cent or 20 per cent. Among natives of India the large mononuclears and transitionals averaged 21 per cent in the apyrexial stage of malaria while healthy natives rarely showed as much as 10 per cent (Stott). Other observers who have made counts of the large mononuclears and transitionals in different parts of the world in the tropics have found the normal percentage in healthy individuals between 3.1 and 6.6 per cent which corresponds very well with the normal count in temperate climates.

**Wassermann Reaction**.—Attention has been called (p. 63) to the frequent positive Wassermann reaction observed in malaria. In countries where syphilis is also common one obviously cannot rely upon this reaction for differential diagnosis.

**Henry's Flocculation Test**—Henry (1927) has shown that the blood serum of a malarial individual is apt to flocculate if mixed with a suspension of melanin extracted from bull's eyes (Henry's reaction). This reaction was first thought to be due to the specific precipitation between antibodies in the serum and melanin (the antigen). However it has been shown subsequently that the reaction is due to the non specific precipitation of the serum globulin which is much increased in quantity during the acute stage of the disease.

Chorine (1937) found that identical results could be obtained by using distilled water instead of the melanin suspension provided the reading is made with the help of a photometer. The melanin therefore must be considered as an intensifier only and although its presence makes the reaction more easily readable to the naked eye it is non essential the reaction actually consisting essentially of the non specific precipitation of euglobulin by distilled water.

Recently a large number of reports regarding this reaction from different countries have been published a number of which emphasize its occurrence of in many cases of malaria. Wright Trensz (1936) Chorine Tyagaraja (1938) and Wolff (1939) have employed the tests successfully. Wolff (1939) emphasizes that although the test is not specific this does not imply uselessness. Wolff has employed in addition a new test a buffer precipitation test which is a further development of Chorine's modification of the Henry test. This buffer precipitation test aims at the precipitation of the euglobulin under the stabilized conditions of the buffer solution arranged in a set of different pH concentrations. For practical purposes a small set of 2 tubes only (pH 7 and 7.4) proves efficient in most cases. For several months he has employed this buffer test and compared it at the same time with Henry's test on 350 sera. He found no disagreement between the 2 tests as regards undoubtedly positive cases but occasionally a disagreement as regards borderline cases. He is still undecided as to whether his buffer test gives better results than Henry's test in such borderline cases.

The test has been recommended as follows (a) in cases of suspected chronic malaria (b) in acute cases where the parasite finding is difficult as a result of the administration

of drugs (c) in the elimination of malarial carriers amongst blood donors and (d) to control efficacy of treatment

Proske and Watson (1939) have also described a protein tyrosine test which they consider valuable in the diagnosis of malaria. The principal reagents consist of sodium sulphate solution sodium hydroxide solution tyrosine standard solution and the phenol reagent of Folin and Ciocalteu. This test when serums are used they assert gives a simple accurate colorimetric reading which obviates the necessity for a photometer which was required by the Henry serodiagnostic test. The procedure is based on the fact that proteins possess a chromogenic property which can be measured quantitatively against the color produced by pure tyrosine in the presence of a phenol reagent. This chromogenic value is constant for a given protein and the intensity of the color produced can be used as a measure of the amount of protein examined. The tyrosine chromogenic index is determined by comparison with standards procured from pure tyrosine. As a result of the examination of more than 2000 normal blood sera these investigators found that the tyrosine index for euglobulin fluctuates between 50 and 80 while that for serum from malaria patients ranges from 80 to 280 or higher. They believed the test to be indicative of the presence of malaria in 97.4 per cent of known malaria cases examined as compared with 81.9 per cent positive thick blood films examined at the same time. They point out that like the Henry test and its modifications this test is non specific but its high sensitivity in malaria may make it a useful adjunct in the laboratory diagnosis of this disease and possibly in the differential diagnosis of other pathological conditions characterized by an increase in serum euglobulin. However the recent work of Schwartzelder and Adams (1941) has shown that while the tyrosine indices were usually above normal in individuals suffering with malaria high indices were also obtained with the sera of a number of individuals suffering with other diseases especially leprosy paresis tuberculosis and typhoid fever. They regard the clinical value of the protein tyrosine reaction in the diagnosis of malaria as little more than supplemental.

In the case of all these chemical tests which are not specific extensive further study must be made of their occurrence in other pathological conditions as well as in normal controls. In this connection it may be mentioned that kala azar has been found to give a positive malarial (Henry's) reaction and wherever these 2 diseases are endemic constitutes an important source of possible error. However the globulin is generally increased in kala azar and unchanged in malaria and the pseudoglobulin is unchanged in kala azar but decreased in malaria while the albumen is decreased in both conditions. Certain forms of splenomegaly presumably of other origin have also frequently given a positive malarial reaction. Hence while these reactions are of considerable scientific interest it would be very unwise to rely upon them alone for the diagnosis of any case of malaria.

Complement fixation has been reported by Coggeshall and Eaton as a specific reaction in malaria in employing *P. knowlesi* antigens and this has been confirmed by Dulaney and Stratman Thomas (1940). The parasites were washed as free as possible of haemoglobin and other blood constituents and dried *in vacuo*. When ready for use a standardized amount was rehydrated with physiological saline frozen and thawed and the supernatant fluid used as antigen.

Dulaney and Stratman Thomas have tested the sera on 83 patients in whose blood malarial parasites were demonstrated. Seventy two per cent gave a positive complement fixation for malaria at some time during the course of the disease. They point out that a positive reaction is probably diagnostic of malaria but a negative one does not rule out malaria. See also page 62.

In a study of the sera from 34 individuals presumably free from malaria 127 were negative and 7 gave weakly positive reactions. In subsequent examinations (1942) they found 24 positive tests in 70 presumably non malarious cases. Coggeshall (1941) states that in induced malaria the test becomes positive about the second week of infection.



## PROGNOSIS

The prognosis in benign tertian and quartan fever is most favorable when proper treatment is instituted sufficiently early, as such infections are practically never fatal in first attacks. Not only may malignant tertian kill in a first attack but it may lead rapidly to a cachexia while the cachexia following upon benign infections is more gradual. It is the tendency to perniciousness which makes us dread malignant tertian malaria as we can never be sure that a paroxysm may not develop cerebral or algid manifestations and these show a very high death rate 25-50 per cent even when treated.

As regards relapses some observers have found that quartan malarial fever is most apt to show this feature and aestivo autumnal the least. Deaderick gives the percentage of cases showing relapses in quartan benign tertian and aestivo autumnal as 65 55 and 45. An important feature of malaria is its invalidating tendency and by reducing the powers of resistance it raises the death rate from intercurrent diseases. Tropical malaria does not seem to affect the native as seriously as it does the *European* but the high death rate of infants among the natives is undoubtedly often connected with this disease.

The prognosis is more favorable in those who are well placed economically and it is the inadequately housed and insufficiently fed that malaria affects most seriously. Coexisting debilitating diseases as tuberculosis syphilis or intestinal derangements render the outlook more unfavorable. Malaria influences pregnancy and lactation and it is the malaria rather than treatment by quinine which is responsible for the tendency to abortion. Statistics vary greatly as to the percentage of fatal cases in malaria. Certain figures from tropical countries give fatal results as occurring in from 2 to 10 per cent of cases while statistics from temperate climates may show a death rate below 1 per cent. Thomas and Sydenstricker (1938), in the treatment of 44 cases of malaria in Georgia found a general mortality of 4.09 per cent but this only occurred in cases of infection with the aestivo autumnal parasite. The mortality from pernicious types of malaria is frequently about 25 per cent. Thomas and Sydenstricker observed a mortality in cerebral malaria of 50.6 per cent. In more recent years of prompt treatment with quinine this mortality was reduced to 11.8 per cent in 76 cases. They regard the reduction in mortality as due to urgent treatment with quinine. In 2 cases that were treated with atabrin the symptoms progressed so rapidly that quinine was substituted as a life saving measure.

The prognosis when complicated by severe blackwater fever is grave.

## TREATMENT

Of all the specifics recognized in medical science quinine stands pre eminent in the treatment of malaria. For many years it has been regarded as the only specific for malarial infection and its use in the treatment of

malaria has been more successful than that of any other drug for the treatment of any other infectious disease. It is doubtful whether any other drug has saved as many lives or relieved as much suffering.

During the past 15 years a number of new synthetic specific remedies have been prepared and among these plasmoquine and atabrin have been particularly employed. It is still too early to express final opinion of their role in the prophylaxis and treatment of malaria but many authorities now appear to consider atabrine to be equally efficient for the average case of malaria and in some respects to be preferred.

**Quinine** \*—The cheapest and most generally obtainable salt has been the sulphate. It is as efficient in the treatment of malaria as any of the salts if given in soluble form. It is soluble in 720 parts of water and contains 74 per cent of alkaloid. When given in pill form it is relatively insoluble and the pills unless freshly prepared or when hard may pass through the alimentary tract without absorption. For this reason it is sometimes advised to give the salt in acid solution made for example by dissolving 5 gr (0.3 gm) in 0.4 cc (6–8 drops) of aromatic sulphuric acid and adding syrup of ginger water to make 4 cc. In order to disguise the disagreeable and bitter taste of quinine which is so objectionable to some people in aqueous solutions syrup of orange or of lemon may be added. Quinine is also rendered less bitter if mixed with milk. Quinine hydrochloride is more soluble (in 32 parts of water) and contains about 90 per cent of quinine. It also may be given in tablet form (sugar coated if desired) and also may be dissolved in water.

When one is in doubt as to the absorption of quinine and also as to whether the drug has actually been taken the urine may be examined as follows. To 3 cc of the urine in a test tube add 5–6 drops of Tanret's reagent heat and filter to remove albumin if present. The presence of quinine is shown by a dense white precipitate on cooling the solution. The precipitate will redissolve on heating. (Tanret's reagent Dissolve 3 grams of potassium iodide 1 gram of corrosive sublimate 2 cc of glacial acetic acid diluted to 60 cc with water. The excretion of quinine is the same whether given by mouth or injected. The drug appears in the urine within 5 minutes and altogether one tenth of the total quantity may be passed in this manner. Howe and Lyon 1943 show the excretion may sometimes be delayed 1–5 hours or not occur.

The League of Nations Malaria Commission (1939) especially recommends the use of *totaquina* a mixture of cinchona bark alkaloids that can be produced at a price well below that of quinine. The new standard preparation contains 70 per cent of crystalline alkaloids of which not less than 15 per cent must be quinine. It has been used very extensively in different parts of the world during the past 3 years and taken as a whole the researches seem to show that *totaquina* for mass treatment is almost as efficacious as quinine in reducing fever and clearing the peripheral blood of parasites.

Among other preparations of quinine recently advocated but of less value and not so widely used are *Tebelin* (Methylhydrocuprein) cupro-o-quinoline or *Cupochin* (paludex) *Eugonin* (euchinin) or ethylcarbonate of quinine *Loquin* containing ethylcarbonate grains 2½.

Woodward R B & Dering W I *J. Amer. Chem. Soc.* 66 849 May 1944 have reported for the first time the total synthesis of quinine.



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has reported for the first time upon the total synthesis of quinine.

*Dosage of Quinine*—The standard method of the National Malaria Committee of the United States of which Bass was chairman recommended the following treatment Give 30 grains of quinine daily by the mouth in three 10 grain (0.65 gm.) doses Keep this up for at least 3 or 4 days or until the acute symptoms have disappeared and follow by 10 grains every night for 8 weeks Where the infection does not present acute symptoms give the 10 grains daily for 8 weeks

Symptoms of cinchonism such as ringing in the ears fullness in the head deafness and dizziness are not uncommon after such doses have been taken for several days or a week For children, Bass recommends  $\frac{3}{20}$  of the adult dose for each year of age so that a child of 5 years of age would receive  $\frac{3}{4}$  of the adult dose Beyond 15 years of age the dose is that of an adult

*Injections of Quinine*—The bimuriate of quinine (dihydrochloride) (72 per cent of alkaloid and soluble in 1 part of water) or the chlorhydrosulphate (74 per cent of alkaloid and soluble in 2 parts of water) have been considered most desirable salts for injections At present partly owing to its extensive use in local anaesthesia and consequent availability bimuriate of quinine and urea has been employed for intramuscular use It contains 60 per cent of quinine and is soluble in an equal amount of water It has been reported to have a slightly greater tendency to produce amblyopia than other quinine salts and should not be used intravenously

The dihydrochloride of quinine may be obtained in 5 gr. tablets especially prepared for intramuscular injection or ampoules containing 9 gr. of quinine dihydrochloride to 2 cc. of saline are on the market One of these injections may be given into the buttocks daily for 3 consecutive days at the maximum

### METHODS OF ADMINISTRATION

*By Mouth*—This is the usual method and is the one to be preferred in all cases where other methods of administration are not necessitated

In the severe forms of malaria vomiting or coma may for the time being make intramuscular or intravenous administration of quinine necessary but oral administration should be resumed in every case just as soon as the condition of the patient will permit When given intravenously the full concentration is obtained in a very few minutes but with other methods this is a matter of great variation

*Intramuscular Injections*—For intramuscular use a soluble salt of quinine as bimuriate or chlorhydrosulphate is dissolved in freshly sterilized saline A 50 per cent solution is commonly used and from 6 to 10 grains of quinine is injected into the gluteal muscles of one side about 3 inches below the iliac crest If necessary the injection may be repeated later on the other side Intramuscular injections invariably produce necrosis but this may be the lesser evil and 1 or 2 such injections will usually kill off the parasites to such an extent that, with the subsidence of symptoms oral administration may be resumed Subcutaneous injections are liable to be followed by necrosis and abscess formation or fibrous indurations, and hence should not be used

It should be emphasized that not only abscess but also sloughing and chronic painful indurations have sometimes followed the intramuscular injection of quinine as

well as tetanus. For this reason unusual care must be exercised in sterilizing the patient's skin as well as the solution and the syringe used in giving the injection. Care must be taken not to inject the quinine into the neighborhood of large nerve or blood vessels. A localized necrosis especially of the muscular fibers occurs after every injection of quinine and such a condition is especially favorable for the development of any microorganisms and especially for the tetanus bacillus. For this reason many clinicians do not believe that more than 1 or 2 intramuscular injections should be given and the drug subsequently given by mouth. Indeed Nocht and Mayer (1938) state that the intramuscular use of quinine amounts almost to malpractice. They prefer intramuscular injections of atabrin which however may be dangerous (see below).

**Intravenous Injections**—All authorities recognize that there are conditions which make intravenous administration of quinine a matter of necessity. The disadvantages of the subcutaneous and intramuscular methods have been pointed out. Intravenous administration may be followed by alarming symptoms or even death. This method finds its justification in the comatose and algid types of malaria where it is necessary to get the effect of quinine upon the parasites in the quickest possible time where delay may mean the death of the patient.

In giving quinine intravenously 10 grains at 1 dose is usually sufficient. Rarely 15 grains may be advisable. A 20 grain dose is certainly dangerous and should not be given. It is usually inadvisable to give more than 30 grains in 10 grain doses in 24 hours. Intravenous quinine seems to be entirely eliminated within 24 hours and most of it within 1 hour. When used in cerebral malaria 10 grains may be given intravenously 8 hours after the first injection if the drug cannot then be given by mouth.

Injections of 10 grains of bismutate of quinine may be dissolved in 10 cc of distilled water. The injections should be made slowly and at least 3 minutes spent over the operation. In algid or collapsed cases Manson Bahr suggests that saline and glucose 5 per cent may be added in amounts of  $\frac{1}{2}$  to 1 pint though he states in comatose cases this does not appear to be of any distinct advantage. Fairley has employed with good results in collapse cases in the Near East campaigns the addition of 10 m of adrenalin  $\frac{1}{1000}$ . The amount of toxin liberated by the rapid destruction of the parasites after intravenous injection may be sufficient completely to paralyze the cardiac mechanism and death may rapidly ensue.

After intravenous quinine a considerable fall in the blood pressure is usually recorded. If destruction of red blood corpuscles occurs blood transfusions of 50 to 100 cc may be of benefit.

Hast (1937) points out that in urgent cases of pernicious malaria one or more intravenous doses may save life. He recommends the bismutate diluted with at least 20 cc salt solution and that the dose should never exceed 10 grains. Great care should be exercised that the solution injected and that the syringe and needle used should be completely sterile.

**Toxic Effects of Quinine**—Quinine except in rare cases of idiosyncrasy is outstandingly non-toxic in effective doses—this is especially important both from the point of view of treatment and of medicinal prophylaxis—and there is little tendency for it to accumulate in the body. In addition to ringing in the ears and deafness the most important untoward manifestations of cinchonism are the very common scarlatiniform eczematous or urticarial rashes gastric disturbances and vertigo.

Impairment of vision may be brought about by quinine and quinine haemoglobinuria is a recognized possibility. Actual deafness and even amblyopia may result and sometimes prove very persistent though rarely permanent. In quinine amblyopia the pupils do not react to light and the optic disc is very pale thus distinguishing the impairment of vision due to the plugging of the retinal vessels by the malarial parasite in which condition the pupils do react to light and the disc usually is a grayish red.

**Quinine Idiosyncrasy**—Fortunately the taking of quinine is well borne by the great majority of persons but in exceptional cases susceptible individuals may develop even after doses as small as several grains (a) severe nausea vomiting or diarrhoea (b) various skin eruptions usually of a scarlatiniform or urticarial type (c) marked ringing in the ears dizziness or deafness (d) impairment of vision (e) dyspnoea and (f) malarial haemoglobinuria. Dawson and Garbade believe an indication of this susceptibility may be obtained by an endermic test.

For the prevention of tinnitus the addition of 10 minims of dilute hydrobromic acid to every 10 grains of quinine has long been recommended.

**Quinine and Pregnancy**—There is hesitancy in giving quinine to a pregnant woman for if administered in large doses it may sometimes cause miscarriage. However unless the malaria is controlled the patient will be apt to abort. For these reasons it is advisable to give the drug in the minimum doses likely to be effective as 3 gr. repeated every 4 or 8 hours for several days. It has been emphasized that a pregnant woman will run more risk of miscarriage and added complications from repeated paroxysms of malaria than from the administration of quinine. Potassium bromide has been suggested to control the ecboic influences of quinine. Clark states that the experience at Ancon Hospital would indicate that quinine can be given with impunity to pregnant women. In malarial subjects quinine after parturition is of value not only in controlling a fever due to malaria but it also favors involution and perhaps aids in the healing of perineal tears.

Quinine is excreted in the mother's milk. This excretion begins some 15 minutes after the drug is ingested and continues for about an hour afterwards. It does not impair the quality of the milk and does no harm to the child.

For the use of plasmoquine compound and atebuin in pregnancy see below under discussion of these drugs.

Quinine taken in prophylactic doses does not interfere with menstruation conception or pregnancy.

**Conclusions Regarding Quinine Treatment**—After some 40 years of observation and study of the subject, the writer believes that for the routine treatment of malaria the principles outlined in earlier years by Bass Stitt and others and employed frequently in the Panama Canal Zone are still most satisfactory.

Immediately the diagnosis is made by the finding of parasites in the blood quinine either the dihydrochloride or sulphate, should be administered in 10 or 15 grain (1 gm.) doses given 3 times daily. Undoubtedly in the milder forms of malaria common in the southern United States as well as in many fever infections the administration of 10 grain doses is sufficient. However in tropical countries where virulent infection is common and there is no idiosyncrasy to the drug the writer believes that it is sometimes safer to give 3 doses of 15 grains each during the first

3 or 4 days of the attack. In certain very severe infections it may be advisable to give even 4 doses of 15 grains each (60 grains) during the first 24 hours of the attack. The 15 grain dose 3 times a day should be continued for 4 or 5 days then for 2 or 3 days more the amount may be reduced to 10 grain doses (30 grains per day). Subsequent to the decline of the fever the individual should take either 15 grains or at least 10 grains at night for 6 to 8 weeks.

Provided that such treatment is initiated *immediately at the onset of the disease and pursued for 8 weeks*, the disease is usually cured and relapses may be largely avoided. If however effective treatment is not introduced and pursued until a later period in the disease the question of relapses becomes problematical. Especially cases of *P. vivax* infection incompletely treated and those in which effective treatment was not instituted until the disease was subacute are particularly liable to relapses for in many such cases the parasites are already lodged in the organs and tissues of the body especially in the spleen and bone marrow where they are further protected from the effects of the drug. With the effective doses mentioned above the temperature should return to normal in less than 6 days and remain so even should the treatment be temporarily stopped. If the temperature does not fall in such a period and remain normal either the drug is not actually being taken or absorbed or the condition is not malaria or some complication is present.

These remarks apply especially to the treatment of individuals of some intelligence and education. Particularly valuable experience of this nature has been obtained by the writer in the treatment of officers who served in the Cuban campaigns during the Spanish American War and subsequently in the Philippine Islands and later in South America especially in Amazonia and in West and Central Africa.

In the experimental treatment of large numbers of natives living under unsanitary conditions many of whom are uneducated and frequently exposed to reinfection and where the actual ingestion of the drug and the amount swallowed is often questionable the conclusions may be entirely different. Indeed some observers apparently have drawn their conclusions especially from work performed largely under such conditions and hence think it unwise to attempt sterilization of all the parasites during the primary attack of malaria. They accept as proved the hypothesis that relapses cease to occur naturally in untreated human beings when the defensive mechanism of the host has managed to overcome the fever and other symptoms which the parasites cause in non immune patients and believe that therefore no attempt should be made during the primary attack to continue treatment in the hope of preventing relapses. If treatment during the first outbreak of symptoms is not successful in preventing relapses they suggest the drug treatment of later recrudescences should be delayed as long as possible in order to permit the patient to acquire some defensive power.

Just when and how such defensive power will accumulate in the individual is not explained nor is it clear. Thompson suggested the



power of acquiring tolerance requires continued exposure of individuals for some 15 years. On the other hand it has been said that the European acquires only a partial immunity. Others have claimed that quinine is more effective if it is withheld until the patient has passed through several attacks of fever.

In the consideration of such hypothetical views the method of treatment of malaria recommended earlier by some of the members of the Health Commission of the League of Nations also requires some discussion. Thus it was recommended that quinine be given only to control the symptoms of the acute attack during 7 days after which the drug should be stopped and relapses allowed to occur the acute symptoms of each relapse being again controlled with quinine. These ideas were based upon the fact that repeated attacks of malaria produced by the same strain of parasite may result in an immunity to infection with that particular strain. It was argued that if in the treatment of the symptomatic attack all the plasmodia are not killed, those remaining multiply until they produce another symptomatic attack that some immunity is acquired during the quiescent period and that this being added to after each relapse a permanent immunity may eventually be acquired. This method of reasoning is also to a considerable extent theoretical especially in regard to when the permanent immunity will result and whether such a condition will actually occur in an individual case. All that we know in this connection is that some natives who are continually exposed to repeated infections in nature may, after considerable periods of time acquire a tolerance against the disease.

As Craig (1937) has pointed out such a method of treatment as suggested would be practically impossible in many instances, and in the case of aestivo autumnal malaria (subtertian infection) in which there is always the danger of the development of pernicious attacks it might be followed by very serious results.

**General and Symptomatic Treatment**—During the course of the fever rest is most important. The patient should remain in bed and be given only liquids. Copious drinks of water or lemonade are advisable. Only fluids, such as broths, should be given for nourishment. In the intermissions of the benign forms one may allow a more generous diet. During the chill the feet should be kept warm and subsequently during the stage of sweating the bed clothing changed. It is important that the patient be not allowed to become constipated and as a laxative 1 grain of calomel in divided doses followed by effervescing phosphate of soda is very satisfactory.

For the nausea sips of an ice cold alkaline mineral water or cracked ice will generally prove effective. In more refractory cases spirits of chloroform or even a hypodermic of morphine may be necessary. Counter irritation to the epigastrium is often a help. Aspirin may be given for the headache, although ice water compresses are generally sufficient. In algid states hot water bottles should be applied to the body. During convales-

cence excesses in food or drink should be avoided as well as fatigue or exposure to wet or cold

It has been suggested that it is advisable during the paroxysm of intermittent fever to wait until the rigor and the hot stage are over before administering quinine. However in general it seems wiser to begin giving the quinine as soon as possible after the definite diagnosis of the disease has been made

In cerebral malaria it has been suggested that an attempt to obviate the accumulation of the parasites in the brain may be made by giving the patient amyl nitrite to inhale with the idea of making the parasites more accessible to quinine. An injection of 5 minims of a 1-1000 solution of adrenalin may also be injected intravenously with the idea of bringing the parasites into more intimate connection with the quinine. In cases with coma or convulsions in which there may be considerable oedema of the brain and increase of cerebrospinal fluid after the injection of quinine has been given it has been proposed that lumbar puncture be performed and the withdrawal of some 20 cc of cerebrospinal fluid. However in those cases in which cerebral irritation is due to multiple punctate intracerebral haemorrhages such treatment has no favorable effect.

Recently Ascoli and his associates in Italy have recommended especially for the treatment of chronic malaria with enlargement of the spleen intravenous injections of adrenalin. He begins with  $\frac{1}{100}$  mgm and gives daily increasing doses— $\frac{1}{50}$ ,  $\frac{1}{25}$  up to  $\frac{1}{10}$  mgm—the last dose repeated daily for 20 days.

In cases of marked malarial splenomegaly it may be advisable to increase the dose provided the drug is well tolerated. In acute cases the adrenalin is given with quinine. Very favorable reports regarding the reduction in the size of the spleen and an improvement in the general condition have been reported. Millettari (1938) and Pizzello (1940) in Italy and Bell in Kenya have confirmed the value of this treatment.

In cases of hyperpyrexia in acute malaria immersion in a cold water bath may be used to advantage.

In chronic cases many physicians recommend arsenic in the form of Fowler's solution or else sodium cacodylate. Some preparation of iron is of course indicated in malarial anaemia.

Dover's powder has been recommended by some as of especial value in symptomatic treatment and it has been repeatedly said that a number of opium fiends of the tropics seem less susceptible to malaria. However the writer has no definite information on this question.

### SYNTHETIC DRUGS

The following synthetic drugs have been widely used in different countries

**Plasmoquine\*** (Praequinine) — Plasmoquine is a quinoline derivative (N-diethylamino-isopentyl 8-amino-6-methoxy quinoline). It is a compound produced by Schülemann, Schönhöfer and Wingler (1924) and is a derivative of methylene blue. It is toxic and should never be given in a dosage exceeding 0.063 gm (1 grain) by mouth daily to an adult. It is supplied in the form of tablets 0.02 gm and 1 of these tablets is to be taken 3 times a day by mouth†. Toxic symptoms frequently appear after these doses consisting of cyanosis without dyspnoea, pallor, nausea.

British Equivalent Pamaquin

† Such large doses are dangerous.

gastric pain headache, dizziness, drowsiness and haemoglobinuria. The symptoms are the result of a true drug poisoning believed to be due to the conversion of haemoglobin into methaemoglobin. In some cases even a methaemoglobinurea results and other symptoms of blackwater fever appear, or jaundice and red cell destruction.

A fatal case of plasmoquine poisoning of this nature was reported by Blackie 1935. Lichtenstein and de Langen 1936, refer to 11 deaths in which plasmoquine was the probable cause.

Formerly the drug was used in parenteral injections, both intravenously and intramuscularly in 1 per cent solution but on account of the toxic manifestations sometimes produced this use is no longer recommended. On account of these toxic effects the League of Nations Committee in its last report, advises that for therapeutic purposes the drug be given only in reduced doses 0.01-0.02 grams per day and some believe that these doses should be given for not more than 5 days. Even in these doses it may produce gastro intestinal and nervous troubles which make it necessary to suspend treatment.

The action of plasmoquine on the malarial parasites is variable. It is relatively ineffective on the trophozoites of *Plasmodium vivax* of tertian and *Plasmodium falciparum* of subtertian, but it has a more specific action against the parasite of quartan malaria. It does not destroy sporozoites. However it has a definite destructive effect upon the gametocytes or sexual forms in tertian and especially in subtertian malaria. The crescents often disappear after 4 days' full dosage. While it frequently destroys the mature gametocytes after they have appeared in the blood it apparently will not prevent the formation of gametocytes since these have been observed to appear in the blood after plasmoquine has been administered for several days\*. Its advantage over quinine is said to be in the destruction of the gametocytes of malignant tertian in a much shorter time.

The conclusions of the experiments by Ciuca (1937) in Rumania confirmed the limited gametocidal action of a daily subtoxic dose of 0.02 gms of plasmoquine in malignant tertian infections. Twenty five to 33 per cent of gametocyte carriers still showed the presence of these after the administration of 5 doses of the drug. Hence it seems clear that no purpose is served by administering a daily subtoxic dose of plasmoquine in order to devitalize the gametocytes. The League of Nations Committee points out that the devitalizing action generally becomes evident 2 days after the administration of the first dose. Hence a single dose of plasmoquine repeated if necessary on the 5th day in cases where the gametocytes are still present will have the same effect.

In earlier years the successful use of plasmoquine in the treatment of blackwater fever was reported by Mühlens and Fischer, Memmi and Schulemann and Cooke and Willoughby. However owing to the tendency for the action of plasmoquine to produce methaemoglobin and to a few reports of cases of haemoglobinuria following its use most authors no longer recommend that it be given in this condition.

Fulton and Yorke (1943) find *P. knowlesi* can be made plasmoquine resistant.

**Plasmoquine Compound**—It was soon observed that it was inadvisable to administer plasmoquine in effective doses on account of its toxicity and that hence the dose must be reduced. Other attempts were made to combine it with quinine in order to improve its therapeutic properties and there was put on the market by *Bayer plasmoquine compound* and later on *chinoplasmin*.

In plasmoquine compound 0.01 gm ( $\frac{1}{6}$  gr) of plasmoquine was combined with 0.125 gm (2 gr) of quinine sulphate while in the latter the amount of quinine was raised to 0.3 gm (5 gr). The usual dose of plasmoquine compound recommended has been 1 tablet 4 times a day for adults with a maximum dose of 6 tablets 1 tablet 3 times a day for children from 6 to 10 years old and 1 tablet twice a day for children under 6 years.

The idea of combining plasmoquine with quinine was with the hope that it would be effective in the treatment of subtertian malaria as the quinine would destroy the asexual forms of the parasite and permit the plasmoquine to exert its action upon the gametocytes. However it was soon found that satisfactory results could not be obtained with such a small amount of quinine and it was then recommended to give an additional 0.3 gm (5 gr) tablet of quinine with each tablets of plasmoquine compound. Low pointed out that the need to combine quinine with plasmoquine points its own moral. Plasmoquine compound was found to give more satisfactory results than plasmoquine alone but the work of Sinton and others showed that still better results could be obtained with more quinine added. As it has been definitely shown that plasmoquine compound is not effective in treating subtertian malaria it has recently been recommended only to employ it in treating benign tertian and quartan malaria.

Manson Bahr states it is comparatively tasteless and produces few of the disagreeable symptoms often associated with quinine therapy. He has found that it is well tolerated by children as well as by pregnant women. Nutter reported treatment of 4 pregnant women with plasmoquine compound without ill effects and it has also been recommended in this connection especially by Muhlens and Fische. From numerous investigations it has now been shown that there is no advantage in using plasmoquine alone for the treatment of the clinical symptoms of an acute attack of any form of infection. In association with quinine or atabrine or administered after one or the other of these drugs, it is often effective in preventing relapses of benign tertian and quartan except in the case of a few particular strains and also apparently of malignant tertian.

Gentskow and Callender (1938) found a marked reduction in the relapse rate in all types of malaria especially benign tertian among United States troops at Panama in those treated with plasmoquine given concurrently with or following atabrine. Covell (December 1943) emphasizes that the chief function of plasmoquine in the treatment of malaria is not the destruction of gametocytes nor the suppression of the clinical attack but the reduction of the relapse rate its greatest value being in the case of benign tertian and quartan infections which are the most prone to relapse.

For simple clinical prophylaxis the Committee of the League of Nations believes it is very doubtful whether the use of plasmoquine is of value and that it has not been proved that better results can be obtained with plasmoquine than with quinine or with atabrine alone except for the purpose of reducing the risk of infection of anophelines with malignant tertian gametocytes.

**Certuna**—The discovery of another new drug with a gametocytocidal action resembling that of plasmoquine was reported by Kikuth (1938) from the Eberfeld Bayer laboratories. This drug certuna (originally called cilonal) is described as a dialkylamino oxyquinolylaminobutan. Kikuth reported that it was at least twice as effective as plasmoquine in preventing the exflagellation of the microgametocytes of *Haemoproteus* of the rice sparrow.

Muhlens (1938) and Missirol and Misna (1938) have reported that this drug in a stable dosage has a markedly destructive action upon the gametocytes of *P. falciparum*.

and also prevents the exflagellation of the male parasite. Muhlens however thought it as toxic as plasmoquine. Sioh (1938) in induced infections of *P. vivax* found that large doses act upon both the fever and the parasites. However it did not produce a cure of these infections for even after large doses (0.05 to 0.07 gm. thrice daily given for 7 days) the patients relapsed clinically and parasitically in 3 to 4 weeks after the cessation of treatment.

All these workers and in addition Sinton, Hutton and Shute (1938) found the drug was well tolerated by human patients and no unfavorable symptoms developed even when doses as great as 0.07 gm. 3 times a day were given. Sinton, Hutton and Shute found that even in doses as great as 0.18 gm. daily for 7 days it appeared to be well tolerated by 5 human patients upon which it was used.

The action of certuna from the few reports mentioned, appeared to be closely allied to that of plasmoquine, and Sinton, Hutton and Shute (1938) reported that it was much less toxic. They therefore hoped that it might be efficient as a true causal prophylactic. However even in doses as great as 0.06 gm. thrice daily for 7 days commencing the day before infection this drug produced no true causative prophylactic action against the dosage of sporozoites of the strain of *P. falciparum* which was used in their experiments both fever and parasites appearing in the blood of all the patients.

**Atebrin di hydrochloride** \*—Chinacrin dihydrochloride—The dihydrochloride of 3 chloro 7 methoxy 9 (1 methyl 4 diethyl amino) butyl amino acridine—This synthetic compound was first prepared by Meitzsch and Mauss (1930) at Elberfeld and was formerly known as 'erion'. It is a yellow crystalline substance which dissolves in water at 40°C in a 7 per cent solution.

Kikuth first reported that this drug exercised a definite action upon the schizonts of all known species of malaria. Its action was first studied upon the malarial parasites in the canary. It was found that in contrast to plasmoquine it exercised no comparable action upon the gametocytes and especially not upon the crescent stage of the subtertian parasite.

It is supplied in tablets which contain 0.10 gm. (1½ grains). The adult dose is one tablet 3 times daily, and it is advised that it should be given on a full stomach. This fact is emphasized, since if the stomach is empty it frequently causes acute gastric pain. As the drug was regarded at first as relatively non toxic, some clinicians have recommended a dose of 6 tablets (0.60 gm.) daily for a few days. It was formerly considered that a 5 day course of treatment (1.50 gm.) was sufficient to cure an average case of subtertian malaria and this is the procedure still generally recommended.

However Manson Bahr (1938) believes that a longer period is necessary as 7-10 days as a primary course. He thinks it is much safer to repeat this course of treatment after an interval of 1 week in order to permit the excretion of the drug from the body. The tablets should be swallowed whole with a drink of milk or water as the taste when chewed is intensely bitter. For children the tablets (0.05 gm.) may be concealed in a raisin or crushed and suspended in honey or syrup. There is not entire unanimity regarding the most advantageous dose for children but the Winthrop Chemical Company (1940) recommends the dose for children of 1-4 years 0.05 gm. (¾ grain) twice daily for 5 days or once daily for 8 days. Children of 4-8 years 0.1 gm. (1½ grains).

\* The British Equivalent for Atebrin Hydrochloride is Mepacrine

twice daily for 5 days or once daily for 8 days. Children over 8 years dosage like that of adults. The drug is usually well tolerated by children but there have been other reports of immediate intolerance of the drug by young children as regurgitation and vomiting. The League of Nations Commission states that relatively larger doses of atebryn are required for successful treatment during infancy and it has often been the custom to administer adult doses to children over 10 or 12 years of age. In this case however effective doses of the drug are already slightly toxic.

Hecht has reported that after the drug is ingested by the mouth it is absorbed in the duodenum and carried to the liver where it is excreted with the bile back into the duodenum to pass once more into the liver with the portal blood. He believes that little atebryn reaches the general circulation until the liver has been saturated since none appears in the urine until the drug has been taken for several days. The diet taken by the patient when he is given atebryn is of some importance since as Peter has shown food containing large quantities of cellulose is apt to absorb it.

**Toxicity**—The disagreeable features reported from the use of the drug in therapeutic doses have been especially epigastric pains a feeling of excitement and light headedness and yellow staining of the skin and urine. The lemon yellow color of the skin which usually does not appear before the third day after the first administration of the drug is not regarded as a toxic symptom and is due to the deposition of the drug in the skin. It is an evidence of the slow excretion of the drug and it has been observed that it is sometimes increased if there is constipation or other intercurrent infections. This pigmentation obviously must not be confused with icterus pernicious anaemia acholuric jaundice or carotinaemia.

Hecht DeLangen and Storm have found from experiments on animals that atebryn given in large doses produced gastro intestinal and cerebral irritation and when administered intravenously it caused a lowering of the blood pressure and had a direct toxic action on the vasomotor centers. Delirium from atebryn has been particularly noted after injections of the drug.

Some observers have found it to act as a cerebral excitant even when given by mouth and this action has been especially reported in native races in the Far East particularly in Asia. In England amongst Europeans such phenomena have rarely been observed. Some of the patients have exhibited mild or transient psychoses while others have become maniacal.

Briercliffe who employed the drug especially during the great epidemic of malaria in Ceylon described nervous and mental symptoms which sometimes caused anxiety. The League of Nations Commission reports that this has been especially the case when treatment was prolonged and when the doses were large or excessive and in those in which atebryn musonate (methyl sulphonate) was given by injection and followed by the oral use of atebryn. However Briercliffe (1937) emphasizes that when atebryn was administered by mouth in Ceylon 0.30 gm was given daily for 5 days and the mental symptoms when they developed appeared towards the end of or shortly after completion of this course of treatment. When intramuscular injections were given only two (each of 0.03 gm were employed) the second 24 hours after the first and the injections were not followed by a course of atebryn by mouth. In some patients the mental symptoms followed soon after the first injection but usually they occurred during the 24 hours following the second injection. Hence in the Ceylon cases the treatment was not unduly prolonged and the doses were not excessive nor were the injections followed by oral use of the drug.

Cruca (1938) who has used the drug extensively has found secondary disturbances in adults very rare but he emphasizes the intolerance of children to the drug.

American negroes seem less susceptible to the toxic action of the drug as Winchester (1938) has employed it during the past 3 years in Georgia and reports no untoward reactions or toxic symptoms among them. However, these studies refer to an experiment in field prophylaxis and the individuals were not under observation in the hospital. Clark has also used the drug for 8 years in prophylaxis in natives in Panama (see Prophylaxis).

Niven and Hodgkin (1936) treated 218 cases prophylactically with atebryn for varying periods up to a year—0.40 gm of atebryn was given weekly in doses of 0.20 gm on successive days. There were 4 deaths in the atebryn series. On two of these autopsies were held and there was found extreme fatty degeneration of the liver. They did not conclude that the degeneration was due to the effects of the drug as this was not clear but that one must accept the possibility that for occasional individuals atebryn administered over long periods may act as a liver poison. Fernando and Wijerama (1935) reported the case of a young man who died apparently from atebryn poisoning 2 months after having received 2 intramuscular injections. All the tissues were yellow and atebryn was detected in the pericardial and pleural fluid.

*Elimination of Atebryn*—The drug is excreted in the urine and is also passed in the faeces. Experimental studies have shown that while a large amount is excreted in the urine a considerable proportion of it is assimilated and retained in the body for long periods. Continued excretion of the drug for as long as 2 months after the last dose has been recorded. A smaller portion of it after being retained temporarily in the organism is eliminated in the form of disintegration products of the original molecule. When present in large amounts the urine itself has a bright yellow color.

Thonnard Neumann and Ledoux found that atebryn is eliminated within 36 days of the ingestion of the last dose. Field Niven and Hodgkin (1936) investigated to what extent when given in frequent small doses the drug is cumulative. They collected samples of urine weekly from approximately 60 individuals who had received 0.40 gm of atebryn weekly for 1 year and examined by ultraviolet light for the presence of atebryn after the preliminary extraction of the drug with amyl alcohol. Only in a few instances was atebryn detected in the urine by this method more than 4 weeks after the cessation of its prophylactic administration. It is of interest to note that beginning about this time there was a rapid return of fever in the cases in the atebryn group which corresponded to the time when the excretion of the drug had fallen to a low level. However, Kebar (1935) found traces of elimination through the urine for a longer period up to the 65th day after the final dose. He also showed that from 50 to 70 per cent of the total quantity of atebryn was excreted in the urine. It may be detected in the urine by rendering it slightly alkaline extracting with ether desiccating and treating the residue with concentrated sulphuric acid. When the drug is present a yellow color is obtained and a distinct fluorescence is visible.

Clinical proof of the absorption and partial accumulation of atebryn in the organism is afforded by the permeation of the histiocytes of the dermis as revealed by the staining of the skin. Foy Kondi and Peristeris consider that part of the atebryn assimilated is accumulated in the cells of the liver and spleen. Analysis by fluorescence appeared to be conclusive in this respect and the quantity retained depended to some extent on the intervals at which the drug was administered. Large doses were eliminated more slowly after being more or less temporarily accumulated.

For the details of the method of determination in blood and urine see Simmons & Centzko, *Laboratory Methods of the U. S. Army* page 235 1944.

in the liver spleen and the reticulo endothelial system in toto. It therefore is evident that unlike quinine atebirin is eliminated very slowly.

*Intravenous and Intramuscular Injections*—The soluble preparation atebirin musonate for injection was dispensed in ampules each containing 0.125 gm dissolved in 3 cc of water. One such ampule was recommended for intravenous and 2 or 3 for intramuscular injections. Atebrin musonate 0.125 gm corresponds to 0.1 gm of the powdered dihydrochloride. The Winthrop Chemical Company of New York also issues ampules containing 0.2 gm and ampules of 10 cc sterile distilled water. Injections up to 0.2 gm have been given in cerebral malaria in some instances without noticeable toxic effects. However in other cases very grave symptoms have followed the use of this drug.

In some instances the injections have been followed by epileptiform fits. Field and Niven observed 2 cases in which convulsions of an epileptiform type occurred soon after the second intramuscular injection. Bardy (1935) treated 50 cases in the Singapore Hospital with 2 injections of 0.375 gm of atebirin musonate. One patient had a cerebral attack which may have been caused by the drug and a second with cardiac lesions died 12 hours after the first injection. Van Heukelom and Overbeek (1936) have also reported epileptic fits in 2 cases treated by atebirin injections which proved fatal. However in a post mortem examination organic cerebral lesions were found in both cases.

There had been no reports of any large number of serious cases of poisoning after atebirin treatment until the extensive use of the drug in the Ceylon epidemic when the British Government purchased atebirin to the value of some \$ 00,000. Briercliffe (1935) in his report upon the epidemic states that probably in rather more than one half per cent of the hospital patients treated with atebirin musonate death has been attributed to the drug. Small children were found especially liable to develop sudden collapse or convulsions after the injection. Four of the deaths recorded were attributed to the drug and of these 4 cases 3 were children.

In view of these facts many observers do not recommend injections of this preparation. It is considered inadvisable that atebirin should be made available to an ignorant public and it is believed it should only be administered under medical supervision.

Manson Bahr has found that intramuscular injections of atebirin musonate may provoke abscess formation.

In regard to the results of treatment with atebirin (by mouth) the research department of the Winthrop Chemical Company on the basis of reports concerning some seven hundred patients with malaria in different parts of the world who were treated with drug has issued a statement summarizing the results. These seem to be consistent in showing that atebirin affects all forms of the 3 common species of plasmodia found in malaria with the important exception of the gametocytes of the aestival-autumnal form. A further exception must be made in the case of all sporozoites.

As regards destruction of sporozoites there is no evidence that atebirin is of much more value than is quinine or plasmoquine though in some instances it is reported that the drug may postpone clinical evidence of the disease for as long as 8 months.



The Malaria Commission of the League of Nations (1937) in its conclusions regarding atabrin states that

the action of atabrin on the gametocytes is of a similar nature to that of quinine. It has no effect from the point of view of devitalization on the gametocytes of *P. falciparum* but the action on gametocytes already present in the blood is perhaps slightly more marked than that of quinine particularly in relation to the gametocytes of *P. vivax* and *P. malariae*. The trophozoites of *P. falciparum* are said to disappear in the peripheral blood after the fourth dose of atabrin in 90 per cent of the cases. For the trophozoites of *P. vivax* and *P. malariae* the drug is thought to have a slightly more rapid action than quinine. However the Commission noted that the difference between the strains of parasites prevented the drawing of uniform conclusions. They thought the action of atabrin on relapses is slightly more effective than that of quinine. The spleen rate in communities treated with atabrin seems to decrease somewhat more slowly than in communities treated with quinine but the effects of the drug appears to continue to be felt for a longer time.

Regarding the action of atabrin on the general condition of patients the Commission reports this seems to be determined by factors which are still not entirely known. The yellow discoloration of the skin is a disadvantage especially during prolonged treatment. Bastianelli (1937) found that the yellow color of the skin usually disappeared 20 days after the treatment was stopped but in a few cases it persisted for a month.

In ordinary cases of *P. vivax* infection the Commission states it is almost immaterial whether quinine or atabrin be used for treatment. For mass treatment where little or no medical supervision is possible the cinchona alkaloids are the most suitable. Medical supervision is necessary if atabrin be used. The administration of quinine preparations and especially of synthetic drugs by the parenteral route should only be resorted to in special circumstances or cases.

In regard to the conclusions of this Commission that the action of atabrin on relapses is slightly more effective than that of quinine especially in the case of benign tertian and of certain strains of malignant tertian there is some difference of opinion. Thus Christophers (1937), in commenting on this conclusion points out that the small differences between recorded results with quinine and atabrin often under different conditions would seem to be very difficult to evaluate.

Gentzkow and Callender (1938) in the treatment of 1696 cases of malaria in the United States Army in Panama with atabrin, plasmoquine and quinine found that atabrin alone failed to prevent recurrences to a greater extent than any other type of treatment. Quinine in large and long continued doses was found to have somewhat greater relapse preventing properties than atabrin in malarial malaria and markedly greater ones in *falciparum* malaria. However plasmoquine given concurrently with or following atabrin had a very definite and pronounced effect upon the relapse rate upon all types of malaria.

Pittaluga (1937) in Spain has also found that the value of atabrin in the prevention of relapses is not so great as was previously reported. He treated 34 cases of infection with *P. falciparum* the usual doses being 0.30 to 0.40 grm daily for 10 days. The relapse rate was 26 per cent.

The Council on Pharmacy and Chemistry of the American Medical Association (1940) reports in regard to the use of atebirin that the spleen of chronic malaria is not affected by it but the acute enlarged spleen yields though more slowly than to quinine. Its relative anti malarial value compared to that of quinine is much debated. It is claimed to act more rapidly and to require a shorter period of treatment. Its prophylactic value appears to be about equal with that of quinine. The intravenous injection is dangerous.

**Other Drugs**—In earlier years methylene blue was considered by some as being the most valuable drug next to quinine the form of methylene blue recommended for use being labeled medicinal. The drug was sometimes given in capsules of 0.10 gm to 0.30 gm (gr 1½ to gr 3) 3 or 4 times a day. Larger doses sometimes cause irritation of the kidneys. The drug dyes the urine and faeces bluish and the patient may even have blue sweat. Its use has been largely discontinued.

The arsenical compound stovarsol or hectine salvarsan neosalvarsan and tartar emetic were formerly suggested for the treatment of malaria. None of them have proved to be of any special value and are not any more recommended for the treatment of acute cases of malaria.

*The arspenamines* according to some reports may relieve the symptoms of tertian malaria temporarily but relapses are common. Recently mapharsen a trivalent arsenic compound formed by the oxidation of any of the arspenamines was enthusiastically recommended for the treatment of tertian malaria.

However Young and McLendon (1940) have treated with mapharsen 10 negro paretics who had been infected for therapeutic purposes with quartan malaria. Each patient received 0.04 gm of the drug intravenously for a period of 10 weeks. Twenty two weeks after the completion of the treatment blood smears from all 10 patients still showed parasites. In their experience while mapharsen did not eradicate the parasites in a single case it nevertheless relieved the symptom. They hence point out that the use of the drug may be dangerous in that it might inadvertently result in malaria carriers being released from institutions before being cured of the infection.

*Sulfanilamide* (paramido benzene sulfonamide) has been recommended for the treatment of malaria. Van den Wielen (1937) in the Netherlands first reported its successful use in the treatment of 2 cases of quartan fever. De Leon also reported successful results in the treatment of 14 cases of *P. vivax* infection.

Hill and Goodwin (1937) in Georgia employed another form of sulfanilamide (prontosil) in 7 cases of aestival autumnal malaria. The prontosil was given intramuscularly in doses of 10 cc every 12 hours for 4 (rarely more) injections. They report the drug was successful in every case.

Chopra and Das Gupta (1938) reported the successful treatment of one case of malaria in a monkey and Gardner and Deeter (1938) the cure of one case of quartan malaria which had been contracted through transfusion of infected blood.

Coggeshall (1938) has also found that sulfanilamide acts as a sterilizing agent against *P. knowlesi* infections in rhesus monkeys a single 1.0 gram dose given by mouth being sufficient. On the other hand this same drug was found to be without effect in the treatment of 30 human cases of *P. vivax* malaria.

Niven (1938) has treated 80 cases of acute malaria with a preparation of sulfanilamide prontosil album (Bayer) 3 grams were given daily in

2 doses At the same time in another series of cases quinine was given in a dosage of 2 grams per 100 lbs body weight of quinine bihydrochloride Each of these drugs was given for 7 days The comparative efficiency of the 2 drugs was judged by two criteria disappearance of fever and the absence of asexual parasites from the peripheral blood In the complete study 80 cases of acute malaria were treated with protosil as compared with 68 cases treated with quinine bihydrochloride It was found that while no toxic effects were noted protosil is not as efficient as quinine in *P. falciparum* malaria and is still less effective in *P. vivax* and *P. malariae* malaria

This drug was also found to be inefficient as a gametocide Mosquitoes were fed on patients with crescents in their blood who had been given protosil for 7 days and these mosquitoes became readily infected Niven concludes that while protosil has some lethal action on malarial parasites especially *P. falciparum* it has no place in the practical treatment of malaria owing to its low efficiency and possible toxicity and relatively high cost

Faget Palmer and Sherwood (1938) also found sulfanilamide ineffective and attended by certain dangers in the treatment of 4 cases of malaria Reid (1940) points out that the use of sulfanilamide compounds in human malaria cannot be recommended until much further carefully controlled experimental evidence is available in regard to human malarial plasmodia

*Summary Regarding Use of Drugs in Malaria*—From what has been said it is obvious that the physician has 2 and sometimes 3 problems regarding the use of drugs in connection with the cure and prevention of malaria First, the treatment of the patient and the cure of the disease by destruction of the asexual parasites Second the prevention of individual infection Third the destruction of the sexual forms in the body and the prevention of the spread of the disease to others

In the treatment of malaria no other drug has been shown to be more satisfactory than quinine In spite of a number of unfavorable statements that have been made regarding the value of the drug in the past few years and the discussion by some of the comparatively greater value of newer synthetic compounds the physician may feel with confidence that by the treatment of acute malaria at the onset of the disease with quinine his patient will be cured if the drug is given in sufficient doses and for a sufficient length of time

Many clinicians with long experience in the treatment of Caucasian patients in the tropics will not agree with the statement that the Commission considers that curative dosages of quinine or of atabrin for treatment of an attack should not be continued longer than 7 days and that treatment for 5 days will often suffice While such advice in regard to atabrin would seem to be appropriate on account of the cumulative action of the drug and its disagreeable features certainly many cases of malaria will be incompletely treated and relapses more liable to occur if quinine is administered for only 7 days If one entirely stops the quinine after 7 days and interrupts giving it (even small doses) for longer than a few days one runs the risk of the parasites invading other cells than the red corpuscles as the endothelial cells where the parasites are apparently more protected from the action of quinine It is in such cases that relapses especially recur

Bass (1937) who has had a most extensive experience with quinine therapy in malaria emphasizes that the parasites that remain after the acute symptoms have disappeared are lodged in the organs and tissues of the body especially in the spleen and bone marrow. As they develop and reproduce they appear in the blood stream. In order to destroy all of these there must be sufficient quinine in the blood at all times to kill them as soon as they enter the circulation. Ten grains a day is about the minimum quantity that will accomplish this and if this dose is taken daily destruction of all the parasites is only a matter of time. The necessary duration of treatment however varies in different individuals. The administration of 10 grains of quinine daily for a week prevents relapses in only a few instances. Quinine therapy must continue for 6 weeks to cure 80 per cent or 8 weeks to cure 90-95 per cent and probably from 12 to 15 weeks to assure cure in 100 per cent of the patients. There is no means of determining whether a brief or a long treatment is necessary in any given case or when the cure is complete.

It is especially in patients that are not treated *early* and given quinine for a *sufficiently long period* that relapses occur. However cured cases are not usually immune and may suffer reinfection at any time.\*

As regards the occurrence of relapses the League Commission found there was no appreciable difference between quinine and acríquine (atebrin).

The problems regarding prevention of infection and destruction of the sexual forms are discussed at length under chemoprophylaxis. (See p 127)

Recent publications from the armed services on the treatment of clinical malaria are in general agreement. The following is abstracted from Circular Letter # 53, Office of The Surgeon General, Washington, D. C. 19 August 1943.

1. *Uncomplicated malarial (patient able to retain oral medication)* The method of choice is to use atabrine alone. Relatively large initial doses are preferred in order that clinical response may be prompt. These larger doses are then followed by smaller maintenance doses.

- Recommended dosage. Atabrine hydrochloride 0.2 gm (3 grs) and sodium bicarbonate 1 gram (15 grains) by mouth with 100 to 300 cc of water (or an equal amount of sweetened tea or fruit juice) every six hours for five doses, followed by 0.1 gm (1½ grains) three times a day after meals for six days (total 2.8 grams in seven days).
- If atabrine is not available use quinine alone as follows: quinine sulfate 1 gram (15 grains) by mouth three times a day after meals for two days, followed by 0.6 gram (10 grains) three times a day after meals for five days (total 16 grams in seven days).
- Plasmochin may be given in connection with either of the above treatments, however its routine use is not advised. If plasmochin is given the patient must be hospitalized and closely observed. The dosage given below should not be exceeded. Plasmochin may be given immediately following atabrine (or with it) or along with quinine in the last days of treatment with the drug. The course consists of plasmochin 0.01 gram (1/10 gr) by mouth three times a day after meals for four days except for the debilitated patient who should receive only two doses a day. Each dose of plasmochin should be accompanied

Rodger (The Lancet 533, April 1944) in a five year survey of 169 cases of malaria in Northern Rhodesia found 77% of infected persons suffered no second attack and of the remaining 27.3% a large proportion was shown to be capable of reinfection and not relapses. It is suggested that malaria can be a non-relapsing disease provided it is treated at once and treatment is completely supervised. Quinine was the drug used. Of the 86 gentian sections, 1 relapsed once. Only five per cent of malaria of any type

by at least 1 gram (15 grains) of sodium bicarbonate. The fluid and sugar intake should be liberal during and for some days after the course. Discontinue *pl. sm. chin* at once if any toxic symptoms appear.

2. *Severe malaria or malaria complicated by vomiting, coma or other serious disorders.* In these cases and whenever a patient cannot retain or fails to respond to oral medication atabrine or quinine should be given parenterally by one of the methods described below.

Recommended parenteral methods are as follows:

(a) Atabrine dihydrochloride 0.2 gram (3 grains) in 5 cc sterile distilled water injected intramuscularly with the usual precautions into each buttock (total 0.4 gram or 6 grains). If necessary one or two additional doses of 0.2 gram (3 grains) may be given intramuscularly at intervals of 12 to 48 hours as soon as the patient can take and retain oral medication. Atabrine should be given by mouth in such doses as to give a total by both routes together of 1.0 gram in forty-eight hours followed by 0.1 gram three times a day after meals for five days (total 2.5 grams in seven days).

(b) Quinine dihydrochloride 0.6 gram (10 grains) in sterile physiological saline 300 to 400 cc (minimum 200 cc) injected intravenously with the usual precautions, especially avoiding speed. If necessary there should be no hesitation to cut down to the vein. This treatment may be repeated in six to eight hours if the situation demands it. When the patient can take and retain oral medication give a complete course of atabrine (preferable) or quinine by mouth as described for uncomplicated cases.

(In coma the intravenous administration of quinine is preferable in the light of present knowledge but it is possible that the intramuscular injection of atabrine is equally effective.)

3. *Clinical Prophylaxis (Suppressive treatment).* (a) The recommended method is to give 0.1 gram of atabrine ( $1\frac{1}{2}$  grains i.e. one tablet) once daily at the evening meal six days each week (total 0.6 gram per week).

An alternative method of suppressive atabrine administration which has been satisfactory in some areas is to give 0.03 gram of atabrine ( $\frac{3}{4}$  grain i.e. one half tablet) once daily at the evening meal six days each week and a dose of 0.1 gram ( $1\frac{1}{2}$  grains i.e. one tablet) at the evening meal on the seventh day (total of 0.4 gram per week).

b. *Quinine.* If no atabrine is available give quinine sulphate 0.64 gram (10 grains) after the evening meal each day while need for emergency suppressive treatment exists (See note below). Then discontinue, observe for indications of malaria and if required give curative treatment as above (par. 2).

*Note.*—There is no drug which in safe doses will prevent mosquito borne infection with malaria. However quinine and atabrine in small doses are useful in suppressing the appearance of clinical symptoms after infection. They are almost equally effective. Such suppressive treatment will enable malaria infected troops to maneuver and fight actively in spite of an infection which otherwise would incapacitate them. When these troops stop taking suppressive treatment many of them may develop clinical malaria and require therapeutic treatment. It may be wise to stagger the terminal point of prophylactic medication so that hospital facilities are not overtaxed when a large force returns from a hyperendemic area.

*Caution.*—The limitation of the supply of quinine is so great that the use of the drug must be restricted as directed in S. G. O. Circular Letter No. 179.

1 Dec 1942. Quinine should never be used for suppressive treatment except in emergencies when atabrine is not available and exceptionally for the very few individuals who cannot tolerate atabrine.

When quinine is available in abundance the writer believes the suggestions given on pp. 114-115 should be considered.

## PROPHYLAXIS

The methods of successful prophylaxis against malaria vary greatly in different regions and depend particularly upon the ecological conditions present and the value and intelligence of the inhabitants preparing a public health campaign.

region with the present state of our knowledge it is often advisable that efforts should first be especially directed to reduce the incidence and severity of the malady for experience has taught us that in many communities with the means obtainable the complete eradication of the disease is often impracticable While in many regions much of the reduction in the incidence and severity of malaria has been due to anti mosquito measures in others the general distribution of quinine and the improvement in the condition welfare and housing of the inhabitants have played an almost equally important role

In regard to any badly infected district a preliminary survey should be made which will include microscopical examination of the blood and determination of the spleen rate of the inhabitants especially of children It is advisable that such examinations should subsequently be made at monthly intervals throughout the year A second important measure for such a community is to arrange for the treatment by quinine of all those found infected In certain rural districts the free distribution of quinine has been almost the only important anti malarial measure that it was practicable to conduct It is advisable to establish an organization that will be prepared not only to diagnose and treat promptly with quinine all those cases with febrile or other symptoms of malaria but also to carry out blood examinations at regular intervals of the entire inhabitants with prompt treatment of all found infected Early diagnosis and efficient treatment of all found infected may not only play a most important part in the reduction of the severity of the disease but when efficiently and completely carried out exercise considerable influence in lowering its incidence

Among the indirect methods of reducing the prevalence of malaria great importance should be attached to measures which aim at the improvement of the economic and social condition of the people their general well being and standard of living The beneficial effects of such measures have especially been illustrated during the past few years in Italy where in certain localities formerly severely malarial the inhabitants have been provided with better dwellings and their standard of living much improved Through such measures and the additional opportunities provided for the distribution of quinine the malaria rate has been very greatly reduced

Education regarding the dangers of malaria and the method of infection also has been of considerable importance in this respect Russell (1941) in an important article has discussed some of the social obstacles to malaria control However the basis of permanent prophylaxis against malaria in a given region must depend particularly upon the destruction of the mosquito and especially of that particular species of mosquito which serves for the propagation of the disease in the region concerned for it is now recognized that the spread of malaria in nature is principally governed by two well known characteristics of *Anopheles* mosquitoes

In the first place every species in spite of tendencies to spread into unusual breeding places is restricted in its multiplication and distribution particularly by an adaptation to certain types of surface water in which alone it can breed in effective numbers and secondly of the species which may be considered potential vectors the great majority are rendered comparatively harmless in nature because their biting habits do not bring them into frequent and especially repeated contact with man

In many regions malaria is the farmer's worst enemy and rural agricultural populations usually have little money to spend on its control. However by encouragement and education even the poorest and most ignorant peasant can often be stimulated to work to protect himself and his family as well as his associates from devastating disease. Under such circumstances any attempt to control malaria must be based on principles of simplicity and economy and as Hackett (1938) has emphasized naturalistic measures offer almost the only hope of practical economy

**Anti malarial Measures**—Three important methods in the prevention of malaria all of which may be combined as was the case in the earlier campaigns against the disease in the Canal Zone region of Panama are (1) Destruction of anopheline mosquitoes and their breeding places (2) protection of the individual from the bites of mosquitoes and (3) quinine prophylaxis. In some instances it may be advisable and simpler to carry on the mosquito warfare without regard to the question of the species of mosquitoes destroyed. In general terms the malarial mosquito breeds in the suburbs of towns or in districts more distinctly rural while the transmitter of the more dreaded yellow fever prefers breeding places in the immediate vicinity of city houses. Bentley noted that with improvement in agricultural methods and utilization of marshy lands malaria tends to disappear due almost as much to the physical improvement and thereby greater resistance of the people as to the destruction of mosquitoes by the draining of the swamps. The resulting greater prosperity makes better food and shelter from mosquitoes obtainable.

**1 Destruction of Mosquitoes**—Such measures should be directed toward both the larva and the fully developed insect.

**Measures against larvae** When practicable permanent measures should be preferred to temporary ones and when agricultural development goes along with drainage or swamps the cost is often repaid. The doing away with mosquito breeding places may be accomplished by filling in pools or by making ditches with smooth sloping sides to carry away the water. These ditches require a great deal of attention to prevent their filling up with tropical vegetation and thereby adding to breeding places. Subsoil drainage with tiled drains is better. Care should be exercised that public works operations do not raise the level of the subsoil water since in malarial or potentially malarial districts this may be most dangerous.

Anophelines tend to breed in sluggishly moving streams or in stagnant pools especially where there is a luxuriant growth of weeds or grass and are not apt to be found in rapidly flowing streams hence the necessity for constant care of ditches and the like to prevent their becoming obstructed by vegetation or silt

*Larvicides* — When filling in or drainage is not practicable the method of oiling the surface of the pool with crude petroleum is often of value

It has been recommended that  $1\frac{1}{2}$  pint for every 100 square feet of surface be used and repeated every 2 weeks In the Panama Canal Zone a preparation called larvicide was especially employed It is a mixture of phenol turpentine and sodium hydrate which is both cheap and rapid in action Mayne and Jackson recommend creosol as the best larvicide In 1 to 1 000 000 parts it is an effective larvicide and even in 1 to 1 000 000 000 it is destructive to young larvae

Winds are apt to blow away the surface coating of oil and it is difficult to oil the surface of a pool filled with grass Wise recommends crude carbolic acid using 1 ounce to 16 cubic feet of water In using any oily larvicide it is well to introduce it along the banks of water collections with a long spout can and mix it thoroughly with a stiff reed broom

Murray (1936) has made an exhaustive study of the mineral oils as a mosquito larvicide His findings indicate that if oil penetrates the tracheae the larvae fail to mature even if the vapor from the oil is not necessarily fatal Anopheline pupae were found very susceptible to oil In selecting the oil it is desirable to secure one that rapidly penetrates the tracheae of the larvae (one of medium boiling range not involatile enough to be markedly viscous and not volatile enough to irritate the larvae and cause their tracheae to collapse and thereby prevent penetration of the oil)

Wafsy (1936) has found an acid oil sludge from the distillation of crude oil to be an efficacious larvicide in the extensive work in Egypt In Panama partial drainage together with periodic use of crude oil on bodies of water has been in progress for some 20 years While it has had limited success in control it has not eradicated infection In Alameda County California the spreading of semi refined fuel oil by air plane all over mosquito breeding areas has been reported to be very successful and that it has not had the disadvantage of killing fish and vegetation which results from the use of crude oil Also it has not been unduly expensive

*Paris Green* — In places where oil is not effective Barber recommended *Paris green* mixed with dust and so used as to form a scant surface deposit Anopheline larvae being surface feeders ingest it and are killed It does not affect *Culex* larvae On account of its ease of transportation and adaptability to weedy places where oil does not penetrate Paris green dust has proved a valuable selective larvicide



The application of insecticidal dusts by aeroplane was first demonstrated by the Army Air Service in cooperation with the Ohio State Experimental Station in August 1921 and excellent results have been obtained in the United States in the distribution of Paris green by this method

Experiments conducted at Quantico Va. showed the effective quantity of Paris green to be 1 pound per acre. Flying at an altitude of 100 feet or less a 25 per cent mixture with powdered soap stone or hydrate of lime was effective. In winds of greater velocity and at altitudes greater than 100 feet a 50 per cent mixture was required. The material cost about 70 cents per acre per season. Road dust sand ashes flour sawdust and other materials have been mentioned as satisfactory diluents. In treating small areas good results have followed the use of a 1 per cent mixture of Paris green in road dust distributed by a hand machine such as the Champion dusters. Griffiths found that a mixture of wet sand and Paris green is apparently lethal to sub surface as well as surface feeding mosquito larvae. Local conditions however determine the methods which will give the best results in each locality. The use of Paris green dust mixture was found to be of little value in Panama not only on account of the difficulty in keeping the mixture dry during the long rainy season but because most of the powder would adhere to the moist surfaces of the vegetation and little of it would reach the surface of the water.

More recently Barber (1936 & 1941) Rice and Mandelkos have improved the method and described a dustless mixture for diluting and spreading Paris green. It is mixed with kerosine and either applied by spreading or else mixed with pebbles or gravel and the latter broadcast over the breeding area. The kerosine is not used in sufficient quantities to act as a larvicide but serves as a vehicle for spreading the Paris green and keeping it afloat. Barber (1940) has found it especially valuable in the campaign in Brazil against *A. gambiae*.

In Italy and in Sardinia Paris green has been especially successfully used. Several large districts including the Roman Campagna and the Pontine Marshes have been made again economically productive after having been abandoned for centuries on account of the enormous numbers of infected malarial mosquitoes. An advantage of its use is that fish are usually not killed in the amounts employed.

A preparation known as *Greenglide* has recently been placed on the market which is said to have an advantage over crude Paris green in that it will float for weeks. It is composed of arsenious oxide 55.37 copper oxide 31.12 water soluble arsenic 1.

Russell and Knipe (1942) have made important observations on the automatic distribution of Paris Green.

*Stoxal* which is a special preparation of formalin or paraform is regarded especially by Roubaud as being superior to Paris green in its destruction of anopheline larvae and at the same time much more harmless to fish. This powdered preparation is of great buoyancy and is so fine that its particles are ingested by the young larvae. It can also be dispersed over great distances by the wind. One centigram of the powder

is sufficient for 1 square meter of surface. It is recommended that the powder be mixed with 50 times its volume of dry sand and the mixture then distributed by hand or shovel into the water. The Stoval then dissociates from the sand and forms a film over the surface which destroys the larvae.

*Copper sulphate* has been found useful for tanks in which drinking water is stored and where algae may be present protecting the larvae. This substance does not destroy the larvae themselves but it destroys their food supply.

**Biological Control**—In the past few years many investigations have been carried on upon the biological control of mosquitoes. The value of shading in the destruction of different species has long been recognized and has been especially employed in the Far East and recently in Cuba (1938). In the latter place however it has been emphasized that it is much more effective to keep shade than to improvise it and that improvised shade is frequently unsatisfactory. Russell has pointed out that in India especially in Assam plants like tarapat, duranta, hibiscus and wild fig have been planted along the banks of streams and where dense shade is produced the transmitting mosquito in that region (*A. minimus*) disappears. The cultivation of the plants requires close supervision but in a number of instances it has proved very effective. Blacklock has also reported the successful control of *A. minimus* by such measures and recommends using similar methods in Africa. Kirk (1937) has found that the planting of a belt of shade trees on each side of rivers and streams in Mauritius to prevent erosion has prevented the breeding of *A. costalis* (*gambiae*). Light shade was found to be almost as effective a deterrent to oviposition as was dense shade. *Eugenia jambos* was found to be the most suitable shade tree. It was believed that the inhibitory action of shade is through the inability of the necessary food organisms for the larvae to develop in the absence of light. Certain plants have been found to be inimical or destructive to the presence of larvae in some instances probably by their mechanical interference with oviposition and restriction of free surface for larval life.

Among these are the various species of *Lemna* and the ferns *Salvinia* and *Azolla*. A complete cover of *Lemna minor* indicates an absence of larvae but the growth varies in extent and density very rapidly. In India a pool with any considerable growth of the rootless *Wolffia arrhiza* is almost completely free from all anopheline larvae as it forms an impenetrable scum over the water. Several species of algae of the genus *Chara* have been reported as destructive to larvae by altering the hydrogen ion concentration of the water. However the hypothesis regarding the value of *Chara* as a plant inimical to the presence of larvae according to the observations of Matheson and Hinman is untenable. In the Philippine Islands dried roots of the genus *Derris* have been found distinctly toxic to mosquito larvae when dissolved in water.

In the Tennessee Valley in the United States the malaria control program for the past years has included further experimental work on the efficiency of various herb

cades upon aquatic vegetation known to harbor *A. quadrimaculatus*. As yet however little information has been obtained concerning the effective poisoning by such plants in water. Recently more favorable results have been obtained by spraying the aquatic vegetation with sodium arsenate applied in liquid form. Hall (1940) has found that the lizard's tail *Saururus cernuus* L. is an emergent aquatic species of wide distribution in the eastern United States. It furnishes conditions very favorable for the production of anopheline larvae and the well developed leafy horizon greatly impedes the application of larvicides. However by the use of powder sodium arsenate 100 lbs to the acre attenuation of this species may be rapidly accomplished.

**Fish**—In large sheets of water and streams that cannot be filled up, and also those that cannot be spread with larvicide such as wells and springs certain natural enemies of the larvae may be introduced, preferably small fish. For a number of years 'millions' (*Gerardinus poecilioides*) a small larvivorous fish which is common in Barbados and Central America was particularly used. Most countries however, possess fish equally efficacious to this species.

In Europe Terni has found the use of such fish as carp and tench of value for larvicidal purposes and these have a food value as well. Also in Europe the goldfish stickle back have been found very destructive to mosquito larvae. Prashad and Hora (1936) have studied anew the larvivorous fishes of India which include predacious species species of commercial importance and small carnivorous species. In Asia and India especially species of *Haplochilus*, *Ambassis*, *Anabas*, *Barbus*, *Trichogaster* and *Nuria* have been found useful.

The top minnow *Gambusia* which has been found effective in the West Indies Hawaiian Islands and the Philippines was later introduced into Europe. Hackett (1938) states that *Gambusia* has now been introduced into every major malarial region of the world. He believes it is a hardier fish than the species *Ledist's reticulatus* of Barbados also known as 'Millions' or any of the other top feeding minnows. It is also the most adaptable, tolerant to wide ranges of temperature, salinity and pollution, easily acclimatized to the tropics or to regions of the temperate zone with hot summers and rigorous winters and transportable over long distances without mortality. The species *Gambusia affinis* is viviparous and produced 50 to 100 young at a birth. It is said that an adult can eat 1000 mosquito larvae a day and it will thrive in dirty water choked with weeds. In the latitude of Italy females emerging from hibernation in May can have 3 broods by September and can give rise to 3 successive generations during the summer. No epizootic disease is known to which it is susceptible. It lives on surface food and is especially attracted by mosquito larvae which are moving and which seek protection in surface vegetation. In one well studied region on the northern Dalmatian coast *Gambusia* alone has in 10 years reduced the anopheline density below the threshold of malaria transmission. In many other areas it has not been completely successful but it has been considered responsible for a significant diminution in the anopheline population. Only in a few places has it been reported a total failure. Many of the objections raised on economic grounds particularly that it would destroy the ova or feed on the young of game fish in certain waters have proved to be erroneous. In the past few years it has been introduced into California where its introduction was formerly opposed on the ground that it might eat the eggs of game fish and even their young in the earlier stages.

Walch has dealt with fresh water carp ponds in the Netherlands. Indes in a somewhat different manner. These ponds are full of submerged water plants and vegetation and breed anophelines in large numbers. In addition to the introduction of carp a species of fish *Puntius javanicus* was introduced which feeds on plants. As a preliminary the edges of the pond were clean weeded. By these simple means alone the breeding of larvae was said to be completely abolished.

*Other natural enemies* of mosquito larvae are protozoan parasites planarian and myrmethyd worms arthropod predators of several orders fungi and plants like *Utricularia*. Native species of such groups destroy many larvae the net result of which is to keep the mosquito population down to its present level. Further knowledge and detailed studies of their life histories may indicate that some of them may in the future be utilized more successfully.

**Measures against Adult Anophelines**—A primary measure of importance has been the destruction of adult anophelines particularly in houses. Sinton and Watts have found an insecticidal mixture composed of 1 part of pyrocyde in 19 parts of kerosine oil which is comparatively cheap and efficacious against anophelines. Watson and Singh (1937) have compared the efficiency of 64 drugs and found them all inferior to this pyrocyde kerosine preparation.

When inside the house mosquitoes may be destroyed by sulphur fumigation 1 or 2 pounds of sulphur for each 1000 cubic feet and with an exposure of 2 hours.

Pyrethrum powder which is set on fire with a little alcohol may be burned using 2 pounds per 1000 cubic feet and an exposure of 4 hours. This does not certainly kill the insect and the stupefied mosquitoes should be swept up and burned.\*

Giemsa's spray is now considered an excellent measure for killing mosquitoes in rooms. The composition is as follows: Pyrethrum tincture (20 parts powdered pyrethrum blossoms to 100 parts alcohol) 480 grams odorless potash soap 180 grams glycerine 240 grams. Before using it dilute with 20 times its own weight of water and spray the walls of the room with a spray pump.

Many commercial preparations have been advocated. One of the more valuable of these for use in a spray is known as Flit. It is a proprietary preparation and is expensive to use on a large scale. For personal use many oils and ointments have been advocated to be rubbed on the hands and face and ankles. Another proprietary preparation known as Skatofax has been employed extensively. Oil of citronella which has also been extensively used as a mosquito repellent sometimes irritates the skin and is often not very effective in keeping away mosquitoes. Insect repellent No. 612 (2 ethylhexaned 1,3) has proved to be an excellent insect repellent. Gesal or Neocid (Dichlorodiphenyl trichlorethane) is regarded by many as the most effective synthetic insecticide.

The clearing away of grass and brush from around houses is sometimes effective, as it exposes the mosquitoes to the sun in which they do not live long.

It is usually stated that mosquitoes may hibernate during winter following infection in the autumn and that cases of malaria in the early spring may be explained by their bites. Examination of hibernating mosquitoes for zygotes has not given in some instances strong proof of this view but such mosquitoes becoming active with a rise in temperature may bite gamete carriers in the house and thus spread malaria.

Swellengerdel (1936) in the study of the transmission of malaria in villages north of Amsterdam found that autumnal anopheline infection does not continue over the winter. Infected anophelines were numerous in January and could be found as late as April but they were no longer

Freon pyrethrum Aerosol issued by the Oua termaster U.S.A. has proved most consists of a mixture of liquid freon (freon 12) with 0.8% wax free pyrethrum oil (freon 2,2-dichlorodifluoromethane). The vapor pressure of Freon reduces the necessary spray pressure.

effective malaria vectors because all of the sporozoites they carried were found, from January onward, to be degenerated

Degeneration of the sporozoites commenced in November and was preceded half a month earlier by degeneration of the oocysts. Fresh infections of anophelines continued to occur until the end of October. After that time the remaining healthy oöcyst continued to mature but new infections were rarely added.

To control autumnal anopheline infection in such areas houses should be sprayed once a fortnight between August and the end of October and in order to reduce the amount of useless spraying this should be limited to the houses most likely to harbor numerous infected anophelines. The treatment of malaria patients prevents anopheline infection.

From the second half of August until the first half of October anopheline infections were found to take about half a month to mature. Later on they took twice as long. Swellengrebel believes about 94 per cent of infected anophelines were destroyed by spraying within the houses.

**Direct Destruction**—The use of a small square of wire gauze on a handle (fly swatter) to kill mosquitoes as they rest on a wall is of great value in keeping the number down in a screened house. The Malaria Commission of the League of Nations has advocated the killing of adult female mosquitoes in houses as being the most practical method of controlling malaria in districts in which extensive antimalarial measures cannot be carried out. In the case of *A. maculipennis* in southern Europe which commonly installs itself within houses and cattle sheds this method has proved of considerable value. Swellengrebel, DeBuck and Kraan have also found it of special value in Amsterdam.

This method is not so easy to apply in many parts of the tropics on account of the construction of the houses particularly those of the natives where mosquitoes have every opportunity of entering or leaving. In screened buildings however those mosquitoes that have fed and are desirous of departing commonly collect on window screens and screened doors where they may be easily destroyed.

Especially in Europe the keeping of domestic animals in the vicinity of buildings such as cattle, horses or mules has a tendency to attract the anophelines away from human dwellings. This is especially the case with those species of mosquitoes which find the blood of such animals as attractive as that of man. Advantage should be taken of this knowledge and to separate the animal houses from the human dwelling when possible.

Many types of mosquito trap have been devised. None of these apparently has come into general use. Manson Bahr however called attention to the fact that with one form of box or wire trap in which there was one trap to each 5000 cubic feet of space in a British barracks room with a capacity of 20 000 cubic feet 65 per cent of the anophelines present were captured the trap being at least 2 to 3 feet above the floor level. Other traps have been employed to some extent in Panama. Their use is limited. In India an automatic mosquito-catching machine known as the entoray has been placed on sale. The principle on which it works is that a mercury vapor lamp is intended to attract the insects within the range of an electric fan which sucks the insects down a pipe and into a box of wire gauze. White and his associates and Watts (1937) in India have experimented with this machine. They point out that while some such machine may yet prove of great value for purposes of research it was not found to be very practical as an anti mosquito measure since comparatively few

mosquitoes were attracted to it unless placed very close to the machine. Large numbers of mosquitoes were stained and released as near to the machine as 10 yards and recoveries of them were small.

**Destruction of Mosquitoes Carried by Airplanes**—The probability of airplanes introducing malaria into a country by carrying infected *Anopheles* from an endemic center should be provided against. Reference has been made to the report that *Anopheles gambiae* was probably carried from Africa and introduced into Brazil in this way. Hence all airplanes from endemic centers should be treated with pyrethrum vapor on departure and on arrival which is efficient for destroying any mosquitoes present (see Appendix p. 1745).

**Drainage and Engineering Methods**—In different parts of the world extensive sanitary-engineering projects for permanent drainage have been carried out with considerable success but in many localities such procedures are not feasible and in others little success has been obtained.

Malcolm Watson (1940) says that on many estates in India as in Delhi much malaria has been man made and that over wide areas of both northern and southern India malaria is entirely due to man interfering with nature by clearing away jungles and streams and exposing them to the sunshine and by draining swamps. By so doing the harmless species have been driven out and the breeding of dangerous ones permitted as 1 *minimus* in the north *A. fluviatilis* or 1 *culicifacies* at different elevations in the south.

Drainage should never be attempted without consultation of capable entomologists, field malariologists and sanitary engineers. Faust (1937) points out that within recent years relief drainage in some malarious areas of the southern United States carried on without the supervision of sanitary engineers or malariologists has increased the breeding places of *A. quadrimaculatus* with a consequent increment in malaria and the outbreak of malaria in previously non malarious areas. These results being based on the information furnished by the State Department of Health of the southern United States from 1933 to 1935. Engineers and health officers have at times not paid a great deal of attention to the practical implications of the findings of the biologists.

With the impounding of a number of reservoirs and preparation of others more refined methods for the prevention of malaria have recently been developed by the Division of Malaria Control of the Tennessee Valley Authority. The student is advised if practicable to visit this plant. Especial attention has been devoted to the preparation of reservoirs before the impoundage effort being made to present a clean water surface after filling. With the successful employment of fluctuation of pool level as an anti larval measure various accessory control procedures have been utilized more extensively. It has been shown that marginal drainage, shore line improvement (drift removal) and herbicide work are essential to secure the maximum results of variation of water elevation.

**Naturalistic Methods**—Hackett, Russell, Scharff and Senior White (1938) have emphasized the importance of naturalistic methods in

effective malaria vectors because all of the sporozoites they carried were found from January onward to be degenerated.

Degeneration of the sporozoites commenced in November and was preceded half a month earlier by degeneration of the oöcysts. Fresh infections of anophelines continued to occur until the end of October. After that time the remaining healthy oöcysts continued to mature but new infections were rarely added.

To control autumnal anopheline infection in such areas houses should be sprayed once a fortnight between August and the end of October and in order to reduce the amount of useless spraying this should be limited to the houses most likely to harbor numerous infected anophelines. The treatment of malaria patients prevents anopheline infection.

From the second half of August until the first half of October anopheline infections were found to take about half a month to mature. Later on they took twice as long. Swellengrebel believes about 94 per cent of infected anophelines were destroyed by spraying within the houses.

**Direct Destruction**—The use of a small square of wire gauze on a handle (fly swatter) to kill mosquitoes as they rest on a wall is of great value in keeping the number down in a screened house. The Malaria Commission of the League of Nations has advocated the killing of adult female mosquitoes in houses as being the most practical method of controlling malaria in districts in which extensive antimalarial measures cannot be carried out. In the case of *A. maculipennis* in southern Europe which commonly installs itself within houses and cattle sheds this method has proved of considerable value. Swellengrebel, DeBuck and Kraan have also found it of special value in Amsterdam.

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Among these naturalistic methods and practices have been mentioned

A CHEMICAL MEASURES—

- 1 The pollution of water by waste and vegetable matter decaying and otherwise
- 2 The changing of the salt content of the water

B PHYSICAL MEASURES—

- 1 Natural filling
- 2 Sluicing
- 3 Flooding
- 4 Fluctuating water levels
- 5 Intermittent drying
- 6 Agitation of the water surface
- 7 Stagnating and setting water in motion
- 8 Muddying
- 9 Shading or exposing to sunlight
- 10 Drying the land by planting trees

With reference to the pollution of water it has long been appreciated that sillage and sewage polluted water usually do not breed anophelines with the exception of *A. sundus* and the experiments of Williamson have shown that vegetation and pollution with green vegetable matter either decaying or otherwise as in herbage packing may be used to advantage in the prevention of breeding of anophelines.

In regard to changing the salt content of water Hackett has pointed out that the Zandrani gates at Via Reggio turned a brackish water lagoon into a fresh water marsh and kept the surrounding territory free from malaria for 200 years. Precisely the opposite procedure was found effective in Durazzo Albania. Here the adjacent marsh was turned into a sea water lagoon and the breeding of *A. elutus* prevented by excessive salt. This was accomplished by reversing the usual automatic tide gate so as to admit the sea at high water instead of excluding it. Within 2 years breeding over an area of 15 square kilometers was reduced to zero.

In regard to physical measures a marsh can sometimes be successfully filled by turning a silt bearing stream into it under the direction of a competent engineer. In sluicing the main principle in the construction of an efficient anti larval flush is that a sufficiently large volume of water should be discharged suddenly at a minimum of once a week. The raising and lowering of impounded water levels at frequent intervals to control anopheline breeding has been successfully applied for some years in the southern United States where the principal vector has been a pond breeder. The proper handling of irrigation water either by intermittent drying or the use of alternating channels is an important procedure in many countries where irrigation causes a malaria danger or a mosquito nuisance. Rice fields present a special problem as in parts of India where the flooding of the rice is entirely dependent on an intermittent season of rainfall and where periodic drying is not applicable. Agitation of the water surface is often effective since mosquitoes require still water in which to lay their eggs and mosquito larvae cannot survive in water which is in constant agitation.

With reference to muddying the presence of silt in suspension is usually fatal to the larvae of most anophelines.

With reference to drying land by planting trees one definite suggestion regarding its value in recent times has been reported by DeBoer in Uganda. Here the trees were planted and grew in the waterlogged land. By this means the water was reduced permitting easy drainage and the character of the undergrowth changed from papyrus to Umbellifers and ferns which produced a heavy shade. The planted trees Eucalyptus, Cassia and Casuarina also have an economic value as timber.

Under biological measures in naturalistic control are especially to be mentioned (1) use of fish (2) the changing of fauna and flora. (Already discussed above.) With reference to changing the fauna and flora



the control of malaria methods which are relatively inexpensive and can suitably be carried out by the peasants themselves. Hackett defines naturalistic methods as the deliberate extension or intensification of natural processes which tend to limit the production of mosquitoes or their contact with man. It is pointed out that direct toxic and mechanical measures of destruction or defense taken against mosquitoes are properly distinguished from measures designed to bring about a change in natural conditions of a permanent or semi permanent kind so as to create a new or relatively stable situation unfavorable to the life and activity of the malaria carrying mosquitoes. This naturalistic control is a field which has been largely ignored by engineers and health officers and unexplored to any great extent except by a small number of biologists.

The naturalistic attack on the mosquito and its breeding places is based on a principle of species sanitation which is that it is not necessary to render water unfit for mosquito breeding in general but only for a particular species proved dangerous in the actual area to be controlled. It is conceived that certain anopheline species are rather more rigidly confined than has been thought to definite types of surface water and if these are rendered unsuitable for breeding purposes the species is not able to resort to other waters except in a sporadic way. Difficulties of course are experienced when a species is found with a much wider range of adaptability than others, as for example the European *Anopheles maculipennis* var. *atroparvus* which is found in a great variety of situations in fresh water and in saline running water and still in rice fields coastal marshes canals and drains from Scandinavia to Italy and from Portugal to the Black Sea.

When an anopheline seems to breed in different types of water in different parts of its range Hackett emphasizes it is always to be suspected that we are dealing not with a homogeneous species but with a number of varieties which however they may resemble one another physically may in fact be separate species with different adaptations and behavior. In the case of such widely adaptive insects as *atroparvus* the problem arises as to the possibility of altering conditions so as to favor a less dangerous but competing anopheline species. It is pointed out that land reclamation in the Netherlands has had such an effect and that in that country as irrigation sweetened the land reclaimed from the sea *atroparvus* has been gradually replaced by the fresh water breeder *messiae*. Yet lack of salt itself is not an inhibiting factor as in other countries and regions *atroparvus* is found breeding in all types of fresh water.

Naturalistic methods aimed at the adult mosquito are as yet based almost entirely on the fact that each species appears to have a range of preferred hosts not necessarily the same from place to place in the presence of which it ignores other accessible sources of blood. If this range includes man the mosquito is usually a vector. If it does not, there may be no malaria at all in spite of the high anopheline density. However man is usually only one of a number of hosts which a given anopheline will readily attack and the same anopheline may well be zoophilic and anthropophilic. It is only by a careful study of the habits of breeding feeding resting mating wintering etc. that a point of attack may be revealed through methods analogous to those which in nature so often restrict multiplication and activities of insect species. Such studies must be carried out locally and may have little validity in another region even when the vector is reported to be the same species of anopheline.

In some instances ease of application of quinine prophylaxis as compared with the more permanent methods of mosquito destruction and screening appeals to the sanitarian especially in the tropics. It is just as easy to give quinine to a man in the tropics as it is in temperate climates but when one considers the propositions of draining tropical swamps and shutting off circulation of air on a torrid night with fine wire gauze in the windows and closely woven mosquito nets around the bed the question is decidedly different. In consequence the tendency sometimes is for the average man to despair of accomplishing anything in the way of mosquito destruction and to resign and to seize eagerly on what may be the inferior alternative that of quinine prophylaxis.

Ronald Ross presented this matter concisely and to the point when he stated that it is not a good policy to substitute a measure which does not exclude infection but is merely extirpative in some cases for positive prevention. From this it will be seen that unless it is clearly recognized that quinine prophylaxis may in some cases extirpate but does not always prevent there might be a tendency to adopt it as a measure and neglect the two proper ones.

As regards the relative merits of quinine prophylaxis and protection from mosquitoes Cella gave the following figures:

Treatment	Infected
Mosquito protection plus quinine prophylaxis	1.6%
Mosquito protection alone	2.5%
Quinine prophylaxis alone	10.0%
No protection at all	33.0%

**Methods of Quinine Prophylaxis.**—Various methods of quinine prophylaxis have been suggested. Castellani and others have recommended 3 gr. per day and a double dose once a week. This method has many advocates. Each advocated 15 grains on 2 successive days weekly. Although quinine will frequently not prevent primary infection one great value of its use as a practical prophylactic is to prevent serious malarial paroxysms and invalidism in men on expeditions in infected localities. For this purpose but many years the writer has employed 15 grains (1 gm.) of quinine given on 2 successive days of the week. While this method did not always prevent primary malarial infection when infection did appear in which symptoms of malaria were present the paroxysms were mild. Apparently the only disadvantage of moment in such prophylactic use of quinine is that it makes diagnosis difficult for it is almost impossible to find malarial parasites in the blood of a man who is taking 30 grains of the drug a week. On our expeditions in the Florida Everglades that was diagnosed as malaria by finding of parasites in the blood was treated with from 30 to 45 grains of quinine during 4 or 5 days and then with 15 grains daily for from 6 to 8 weeks. On none of the writer's expeditions in the tropics has any member of the expedition succumbed to malaria though one member who was attacked to an expedition in which he remained longer in the Amazon and did not take quinine died of malaria and other complications.

For the treatment of porters or carriers found to be infected it is recommended that 15 grains (1 gm.) be given on 2 or 3 successive days of each week, the course to be continued for 3 months. Development of paroxysms in such individuals due to a reduction of their immunity as a result of the quinine treatment was not observed.

**3. Chemo prophylaxis.**—During the past few years there has been much discussion in regard to the value of quinine and the new synthetic compounds in the prophylaxis of malaria. After considering the experimental evidence including the work of James Yorke and Macne etc. the League of Nations Commission in its report published in 1937 concluded that while there is some evidence to suggest that atabrin in large but harmless doses and plasmoquine in doses of a toxic nature may exert some degree of true prophylactic effect under certain conditions and with certain strains of *P. falciparum* it is difficult or impossible with our

Hackett quotes as the best known example of the application of this measure that seen in the 'hygienic exploitation' of the fish ponds of the Netherlands East Indies (Overbeek and Soker, 1938). The cultivation of the marine fish called *Chanos chanos* in Java is of considerable dietary value and economic importance.

This fish is cultivated in artificially constructed salt water ponds in which *A. ludlowi* (*sundat us*) breeds in association with floating algae consisting of *Enteromorpha*, *Spirogyra* and other species, the fish breeders being firmly convinced that these algae are necessary for the cultivation of the fish. In conjunction with the fishermen's departure, the bottoms of the ponds were laid dry for a couple of days at least once a month followed by filling up the pond with fresh sea water. During the draining period the fish remained in side ditches of the ponds built for the purpose. The object of the procedure was to kill the top algae by drying and to permit the development of the blue algae which grow on the bottom while the pond is drying. These blue algae remain much longer at the bottom of the ponds and do not rise to the surface until they have grown into a thick layer. It is upon the blue algae that the fish feed. The algae rise to the surface in broad sheets and compact masses and no mosquito larvae can penetrate these. By the introduction of larvivorous fish into the pond any larvae that might be present are eaten. The result of these measures which have been carried out around Batavia has been the practical abolition of malaria in the areas so treated. Boyd (1939) points out that the control of anophelines in the fish ponds of Batavia is the most striking example of known possibilities of this nature in the destruction of the anopheline species.

**2 Mechanical Protection of the Individual**—The ancient Egyptians were according to Herodotus acquainted with the use of the net to protect during sleep against the bite of gnats and it is stated that Emin Pascha always carried a mosquito net and never suffered from malaria. He thought that the cause of malaria was too large to go through the net.

The house in endemic areas should be thoroughly screened with copper wire screens which should have 18 meshes to the inch. Mosquitoes can pass through a 15 mesh screen. Screen doors should always open outward and close automatically with spring hinges. Double screening of doors is desirable.

However it is almost impossible to screen a ship's hatches effectively. Then too the screening of fan intakes and ports interferes with free circulation of air thus adding to the discomfort of the heat of the tropics. As malarial mosquitoes bite chiefly toward evening one should not expose himself after sunset. Houses should be far removed from native habitations. Mosquitoes prefer the lower floors of a house so that the upper stories are preferable for sleeping. Mosquito nets at night with protection by veils for the face or coverings for the hands and ankles and mosquito boots especially when going out of the house are well known measures.

Even when mosquito nets are intact and well tucked in there is the danger that a person sleeping on a narrow cot is apt to put his bare arm or leg against the net in which case the mosquitoes readily bite the skin presenting at the open spaces. Oil of citronella applied to the skin is often used to keep away mosquitoes but it is not always effective.

Stewart These medical officers made their observations on three companies of United States Army engineers employed in mapping Panama. Quinine prophylaxis was compulsory under strict supervision one gram of quinine was given each soldier daily in tablets or capsules before the evening meal. The men worked and were camped in intensely malarial places. The investigators found that of the 225 men who were engaged for 4½ months in mapping and who were taking one gram of quinine daily only 14 or 6 per cent showed clinical symptoms of malaria. Only after the quinine was discontinued did the actual amount of infection in the command become manifest through the appearance of symptoms of malaria in 106 persons or 47 per cent. The troops in Panama were supposedly later repeatedly exposed to bites. This work conforms with Yorke and Macfie's conclusions that *experimentally* quinine does not prevent malarial infection but that continued use of the drug for from 10 days to 2 weeks after exposure to the insect bite always prevents any subsequent malarial paroxysm. McNabb and Stewart conclude that under field conditions in a hot climate where men are undergoing physical hardship 1 gram of quinine will prevent the development of symptoms of malaria in men exposed to infection and will keep them on duty and that although quinine prophylaxis will not prevent infection it has great military value since it will enable troops to accomplish a mission in a malarious region. These observations also demonstrate that although quinine in the dosage given did not prevent malarial infection in 47 per cent of the persons concerned the fact that over 50 per cent of them lived and worked for 4½ months in a most malarious region without developing symptoms of malaria even after discontinuing the quinine indicates that even though infection may not have been prevented a considerable number of persons must have been rid of the infection almost immediately after its occurrence. Craig (1940) though he believes that malarial infection cannot always be prevented even though as much as 1 gm. of quinine is taken daily for prophylaxis also believes that it is impossible that over 50 per cent of a command could escape the development of clinical malaria under such conditions had quinine not been administered.

Simmons (1939) who has had a wide experience with the Army in Panama likewise emphasizes that there appears to be no doubt concerning the value of the prophylactic use of quinine as a military measure as it can be used to prevent the occurrence of clinical attacks of malaria among troops while on duty in the field. It is his opinion also that the prophylactic use of either quinine or atabrin prevents an unknown proportion of infections. Callender and Gentzlow (1938) have also thoroughly demonstrated these facts in troops on field service.

The value of the use of quinine as a prophylactic measure for troops in the field during the World War has also been especially emphasized by the report of the late Sir William Fletcher. The British Army disembarked at Salonika in the autumn of 1915 and although plans were made for quinine prophylaxis against malaria they were not executed. In 1916 there were some 60 000 cases of malaria in a force of about 115 000 men. In the autumn due to malaria there were not more than 20 000

present knowledge to rule out the possibility that this action may be upon the schizogonic forms of the parasite in the red blood corpuscles rather than upon the sporozoites or upon an intermediate stage between sporozoites and trophozoites. With regard to *P. vivax* they conclude up to the present time no drug is known which, when taken in harmless doses during the period of infection will effectively destroy the causal organisms of malaria (sporozoites) before they are able to continue their life cycle in the human host.

In regard to the action on the gametocytes, this Commission has concluded that neither quinine nor synthetic drugs (nor even plasmoquine) administered before the appearance of gametocytes (especially those of *P. falciparum*) in the blood are capable of invariably preventing their formation. Both atabrin and quinine have an appreciable action on the gametocytes (already formed) of benign tertian and of quartan similar to their action on the trophozoites in the blood stream. Plasmoquine has a specific action on the already differentiated gametocytes of malignant tertian since it destroys or devitalizes them to such an extent that even under the influence of a very small dose of the drug (0.05 gm) those gametocytes remaining in the blood become in a few days incapable of infecting mosquitoes. A few experiments such as those of Strickland and Rov with atabrin, show that it may prevent the gametocytes of *P. falciparum* from developing during the time the drug is fully present in the blood. However, Kingsbury (1935) has found that the viability of subtertian gametocytes of *P. falciparum* is little affected even by atabrin musonate treatment. Five out of 6 crescent carriers on whom anophelines were fed at periods from the fourth to the tenth day after the commencement of treatment proved to be still infective. Also, Field (1938) found that atabrin musonate even after 2 injections has no advantage over quinine as a *falciparum* gametocide. In some experiments performed at Forpé the Italian observers found that atabrin exerted a partial action in bringing about the disappearance of the gametocytes of *P. falciparum* already present in the blood. However the action of atabrin even of injections of atabrin musonate on fully formed gametocytes of *P. falciparum* is evidently very slight.

While then under experimental conditions the sporozoites themselves injected by infected mosquitoes in man are not destroyed by any of these drugs, nevertheless in actual practice in the tropics the systematic taking of quinine by an individual in many instances prevents the onset of symptoms of malaria and the individual instead of being in bed and incapacitated for work is able to continue his daily occupation. The value of quinine as a prophylactic in field expeditions has been repeatedly demonstrated even though such value is only relative. A great responsibility rests upon the practicing physician as regards the prevention of malaria for in the proper treatment of initial malarial infection we possess one of the most valuable of all prophylactic measures.

*Prophylaxis in Armies*—A most important piece of research in respect to quinine prophylaxis in the field was carried out by McNabb and

Linnell and several other observers including the writer have found that the giving of 5 gr of quinine daily in badly infected districts is frequently not satisfactory for prophylaxis. Such amounts sometimes only prevent the onset of an attack for a variable period of time. Experimentally it has been found that persons who were infected by mosquito bites 2 or 3 times weekly and who took each day a small dose of 0.30 gm (5 gr quinine bihydrochloride) often had mild attacks of malarial fever between 10 and 14 days after the first infection but the attacks lasted only from 2 to 4 days. Then they remained free from fever for about 2 weeks when another minor attack occurred. A few days after ceasing to take the daily prophylactic dose they developed severe attacks with fever and many parasites in the blood. Other persons infected in the same way but who took as a prophylactic only 1 gram of quinine once a week had more severe and more frequent febrile attacks than the persons who took daily doses of 0.30 gm.

On the other hand in experiments in Italy (1937) in which quinine varying from 0.4 to 0.6 gm (6-9 gr) a day or 1 gm (5 grs) twice a week was taken and the individuals were subsequently bitten once or several times by infected mosquitoes the occurrence of an attack was prevented during the whole course of treatment which sometimes lasted throughout the whole malaria season. In some cases the treatment was successful in preventing any manifestation of malaria. Only in a few instances did it fail.

Different results regarding the value of prophylaxis are obviously partially explainable upon the different susceptibility of individuals in different communities and countries and the prevalence and high or low infectivity of the mosquito hosts in the locality as well as the extent of exposure to their bites.

**Plasmoquine and Atebrin with Quinine in Prophylaxis**—Under the discussion of treatment with quinine and synthetic drugs it has been emphasized that true chemo prophylaxis can sometimes be obtained with plasmoquine but only with such doses as are dangerously near the toxic limit and such doses cannot be recommended for residents in malarious countries.

It was also hoped that atebrin would prove successful as a prophylactic particularly on account of the fact that some of the drug is retained in the body for considerable periods. Nevertheless the drug given in 0.30 gm doses over a period of 5 to 7 days does not prevent penetration of the sporozoites into the tissues though in some instances it does delay the onset of the fever for as long as a month. For prophylaxis with atebrin the League of Nations Committee recommends a dose of 0.1 gm (3 grains) for adults administered twice a week during the malaria season.

A great many field experiments have been performed during the past few years in which the value of quinine and atebrin for prophylactic use has been studied.

Clark and Komp (1939) have carried on observations on malaria in Panama for 8 years. In the last report *compare results in the treatment of two groups of inhabitants*. In one group consisting of the individuals found positive for malaria by the monthly service atebrin (0.10 gm) was given 3 times a day for a period of 5 days followed by plasmoquine simplex (0.01 gm) twice a day over a succeeding period of 5 days. In the second positive group quinine sulphate (15 gr) was given daily for 5 days and during the following week plasmoquine (0.01 gm) twice a day for 5 days. Clark reports that the experiment has been equally good with quinine or atebrin provided the same degree of attention was given to the administration of the 2 drugs.

men in the line. In January 1917, since antilarval measures on a large scale were not expedient for the protection of an army, quinine prophylaxis was rigorously applied. A special anti malaria service was created and the urine was tested with Tanret's reaction to see that the quinine was actually being taken by the men. The result of treatment was successful. Whereas in 1916 the malarial peak in September was reached with 8 000 primary cases (in 4 divisions) in 1917, with 8 divisions the peak did not exceed 1 000 primary cases. In 1916 there were 379 deaths from malaria in 1917, 71, and in 1918 54. Tanret's reaction showed that only about 15 per cent of the men were taking their quinine in 1916 and by 1919 nearly 100 per cent were taking it.

Wenyon at the British symposium on malaria in war (1939) states that he is definitely of the opinion that prophylactic quinine does prevent actual attacks of malaria though it does not prevent infection. During the World War in Macedonia once or twice the experiment was tried of withholding prophylactic quinine in the British Army for a week or two from large groups of men. This was followed by such increases in the sickness rate from malaria that a rapid return to the prophylactic dose was made. Regarding mosquito nets he states that these did more to prevent infection in Macedonia than all the other prophylactic measures together.

It has been suggested that in quinine prophylaxis there may be the possibility of producing an immunity to quinin on the part of the parasites which have been introduced by infected mosquitoes and held in check by the smaller prophylactic but not curative doses of quinine. Later on when the quinine prophylaxis is discontinued the parasites may begin to multiply vigorously and perhaps may show an immunity to quinine. In this connection an instance has been quoted in which 398 marines served in 1906 for about one month on the Isthmus of Panama during which time they were given 9 grains of quinine daily as a prophylactic. During this month there was only an occasional case of malaria among the men. At the end of the month 98 of the original 398 returned aboard ship and sailed for the North. Two days later 20 cases of malaria developed followed the next day by 53 and the day following that by 45. The medical officer then resumed 10 grain prophylactic doses for those not down with malaria but notwithstanding this there were 215 acute malarial paroxysms some of them of pernicious type among the 298 men. It was noted that these men did not respond satisfactorily to quinine treatment even when the drug was administered intramuscularly.

It should be noted in this experience that during each month that the men were taking even relatively small doses of quinine only an occasional case of malaria occurred among them and that as soon as the quinine was stopped cases of malaria began to appear among them. Had quinine been continued in larger doses and for longer periods of time it seems probable that not so many attacks of malaria would have developed subsequently. The danger of incomplete treatment have already been emphasized.

Stott's observations in India regarding quinine prophylaxis (often quoted) were not favorable. Among native Indian troops he gave one group (3931) prophylactic quinine while the other (3906) did not take quinine prophylactically. He continued this experiment 1 year giving 15 grains 3 times weekly for 5 months and 10 grains 3 times weekly for the remaining 7 months. Those taking quinine gave 170 primary admissions while those not taking it gave 19 (43.2 per thousand strength for the former as against 45.8 per thousand for those not taking quinine prophylaxis). In experiments of this nature one cannot be sure that the Indian native troops actually took the amount of quinine regularly that was prescribed in all probability they did not. The experiment merely demonstrates the difficulty in prophylaxis with native Indians.

the onset of a malarial attack for as long as 33-37 weeks after experimental infection (James and Shute) Atebrin can be generally more easily dispensed for mass treatment in rural communities and the time of treatment is much shorter

The physician must often decide when to employ individual prophylaxis While it is true that where only benign malaria exists or where there is satisfactory means of carrying out diagnosis and treatment quinine and especially atebrin prophylaxis is not to be recommended yet in intensely malarious districts with heavy infections of natives it is advisable for the European when actually exposed to infection to take from 5 to 15 grains of quinine every day in tropical regions where malignant tertian is a menace Many experienced observers believe that it lessens the danger of blackwater fever attacks Stitt advised the medical officers of the ships of the U S Navy in tropical regions not to use quinine prophylactically on board ship or in shore dispensaries since they were in a position to recognize readily and treat the onset of malaria and to carry out more or less efficiently mosquito protection methods However on military expeditions or exploring trips in tropical or subtropical countries he also believed it is the only practical method of keeping a force efficient Of course one should first of all insist on the use of mosquito nets as important in protecting from malaria as well as from yellow fever dengue and filariasis Every soldier should be provided with an individual net

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The disturbing feature of the work was the number of relapses that occurred with either form of treatment.

Nevertheless the method of control adopted has been successful in reducing to the vanishing point clinical illness from malaria. The parasite rates during the past year were the lowest so far recorded. The low rates were believed to be caused by a combination of the normal decline in rates and the treatment of all positive cases. No toxic results followed the use of atebtrin in the doses given. Clark and Komp however point out that they have not been successful in completely eradicating the disease and that it is extremely doubtful whether under the conditions in Panama such a result will be possible. The ever present mosquito, the newly arrived carrier and the year long transmission season all conspire to prevent complete success but undoubtedly much has been accomplished in reducing the ravages of the disease.

Winchester (1938) has employed atebtrin in Georgia for the past 3 years. In 1936 all persons (Negroes) found harboring malarial parasites were given a 5-day course of atebtrin (300 mgm a day for adults). These individuals were then placed in one of 2 groups referred to as the prophylactic group and the control group. The prophylactic group received 0.05 gm ( $\frac{1}{4}$  grain) of atebtrin a day beginning May 15th and continuing until the end of October. The control group received no medication after the initial treatment in the spring. In 1937 (the following year) all persons showing the presence of malarial parasites at the spring examination or giving a history of chills and fever during the previous 6 months were treated with a 5 day course of atebtrin. After the beginning of the experiment only 10 individuals in the prophylactic group ever showed parasites in the peripheral blood at any examination. In the control group in 1936 there were 49 cases of clinical malaria and in 1937 28 clinical cases. Winchester regards the results as so encouraging that a further trial is warranted. No untoward reactions or toxic symptoms due to atebtrin were observed.

Field Haven and Hodgkin in the Malay States (1937) made a comparative study of the prevention of malaria in the field by the use of quinine and atebtrin. In one group on two estates 0.4 gm of atebtrin weekly was given in 2 doses 0.2 gm on successive days. The dosage for children was proportionate to age.

To a second group on each estate 0.4 gm of quinine dihydrochloride daily was given as sugar coated tablets each containing 0.2 gm. The dosage for children was proportionate to age. To a third group on each estate 2 tablets of an inert substance designed to resemble atebtrin in appearance was given. This third group served for control. The prophylactic treatment both with atebtrin and with quinine effected in contrast to the control group a marked reduction in the number of malarial attacks. The reduction for the last 6 months amounted almost to elimination. The effect of the prophylactic atebtrin on the malarial incidence and on parasite rates was somewhat more potent than that of the prophylactic quinine. However there was a rapid return of the clinical evidences of malaria when the administration of the drugs was suspended. The safety of prophylactic atebtrin was not established by the observations made but it appears that the risks as well as they could be determined were not of high order. In many tropical countries mass treatment must very frequently be carried out without proper medical supervision of individual patients and then quinine distribution may be preferable. Where the cases under treatment can be carefully observed many clinicians advocate the use of atebtrin. In addition to the favorable reports of its use quoted should be noted those of Bispham (1938) in the southern United States who found 4  $\frac{1}{2}$  grains a week for 4 weeks satisfactory for prophylactic use in the Civilian Conservation Camps. Hill and Goodwin (1938) in a highly malarial region in Georgia U.S.A. found only 1.8 per cent of clinical cases of malaria in a group treated with 0.1 gram of atebtrin 3 times a week as compared with 5.6 per cent of a group given 10 gr of quinine daily and 31.7 per cent in an untreated group. Over a two year period the parasite index of the community was reduced from 17.0 to 0.3 per cent.

A review of other recent literature on the comparative value of quinine and atebtrin as a prophylactic reveals there is not unanimity of opinion as to which drug is more valuable. However atebtrin will often prevent

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1859 Other American physicians during the next 10 years described the disease from various other Southern states Veretas noted the presence of the disease in Greece in 1858

It is rather remarkable that the disease was not noted by so keen an observer as Torti if it existed in his time and Manson pointed out that it is strange that it should not have been recognized in India if it had existed there prior to recent times Its recognition in Africa and the discovery of its prevalence there has been of comparatively recent occurrence and hence this has suggested to some that the disease had been recently introduced into Africa There have been two explanations given of the recent greater prevalence of the disease in Africa and in other tropical areas where malignant malaria prevails extensively which are (1) that there has been a great influx of susceptible Europeans or other foreign people into such areas during the past 20 or 30 years and (2) that the more frequent and effective dosing of malarial patients with quinine is responsible for some of it

**Geographical Distribution**—It is in tropical Africa that the disease is of prime importance as a cause of death and invaliding It prevails chiefly in West Central and East Africa from about 12° N to 1° S latitude in which regions *P. falciparum* infections are widely distributed It is most prevalent in Europeans on the West Coast of Africa from Senegalia to Quanza and also in the Congo and in the deltas of the Niger and Gambalia Rivers On the East Coast of Africa it is likewise widely spread especially along the Zambesi and in the vicinity of Lake Nyasa It has been reported though less commonly on the upper Niger in Tanganyika Territory Uganda in North and South Rhodesia in Abyssinia and the valley of the Upper Nile It is also common in some parts of Madagascar It is less frequent in Northern Africa although a considerable number of cases have been reported from Algeria It is unknown or very rare in Egypt a country where malaria is very rare in Europeans In India it occurs in Assam and a number of other provinces and Stephens states that in the Duars (Bengal) he saw more cases in a fortnight than he had seen in the same time in Africa Krishnan (1938) in an epidemiological survey of these provinces in 1936 found it especially among the Bengalese of the tea gardens It is found also in Burma and northern Siam in the Province of Yunnan in China in Formosa and the Malay Peninsula It also occurs in the very malarious islands Java and New Guinea and in the Netherlands Indies particularly in immigrant Japanese fishermen especially during the first few months after their arrival

In Europe it occurs chiefly in southern Italy Sicily Sardinia and Greece Bulgaria and Albania Blackwater fever was frequently noted among the British forces in Macedonia and Palestine during the World War It is common in Central America and the West Indies and northern South America especially in the regions of the Amazon basin in Brazil

In the United States it is chiefly found in the most malarious sections of Arkansas Mississippi Louisiana Texas Alabama Georgia Florida and South Carolina It would seem that it is becoming more rare in the Southern states As a result of anti malarial measures among the Americans working in the Panama Canal Zone it has almost disappeared

## Chapter II

# BLACKWATER FEVER

### DEFINITION AND SYNONYMS

**Synonyms**—Haemoglobinuric fever malarial haemoglobinuria haemorrhagic malarial fever French *Fievre bilieuse hemoglobinurique* German *Schwarzwasserfieber*

**Definition**—Blackwater fever is a disease the etiology of which has been disputed but there is now general agreement that it is definitely connected with attacks of malaria or a continuous malarial infection. It is prone to affect the Caucasian long resident in parts of the globe where malignant tertian is rampant. It is rare in Negroes but affects among other races Hindus Arabs and Chinese particularly. Blond whites seem more susceptible than brunettes.

On the basis of lowered integrity of the red cells, usually by reason of repeated attacks of malaria and following the administration of a dose of quinine or as the result of refrigeration, excessive exposure to the sun or great fatigue there may occur acute extensive lysis of the red cells with liberation of haemoglobin into the blood stream. Clinically, a prostrating chill of asthenic type frequently occurs, associated with early jaundice and the passage of porter colored urine—haemoglobinuria. There are certain features of analogy with this complication of malaria and the different forms of paroxysmal haemoglobinuria. Splenomegaly definitely attributable to malaria may constitute the outstanding clinical difference between blackwater fever and other forms of haemoglobinuria.

Intravascular haemolysis associated with haemoglobinaemia and haemoglobinaemia occurring in a patient with chronic malaria may be said to constitute the essential basis of blackwater fever.

**History**—It is remarkable that physicians failed to recognize this striking condition until towards the end of the 19th century. It was first described by Lebeau and other French naval surgeons in Madagascar between 1850-60. This late recognition was doubtless due to confusing it with bilious remittent fever and yellow fever. Even after the clinical picture was well recognized disputes as to the nature of the coloring matter of the characteristic urine were frequent some considering that the dark color which we now know to be due to haemoglobinuria was due to haematuria or that the color was due to bile pigment. Blackwater fever must have been the condition referred to in medical literature of the period 1850 to 1870 under the names *Fievre bilieuse haematurique* 'haemorrhagic malarial fever' and *febris remittens haemorrhagica*. It was first described in the United States by Cummings of Louisiana in

the parasites of the latter species not appearing in the blood. However DeLangen and Lichtenstein (1936) and Foy (1938) have observed cases in which only the benign tertian parasite was present. In Foy's cases in 33 per cent *P. vivax* was present, in 47 per cent *P. falciparum*, 14 per cent mixed *vivax* and *falciparum*, and in 6 per cent pigment alone was seen. Other observers including Manson Bahr (1936) believe that the subtertian parasite is the one almost invariably associated with blackwater and that if the blood is examined at the right stage the ring forms of the parasite may be demonstrated in the blood before haemolysis has occurred, but that during the process of haemolysis the corpuscles containing the parasites are broken up and destroyed so that they can no longer be demonstrated. Gates and James in Panama have also given important evidence definitely associating the subtertian malarial parasite with blackwater fever and James has produced blackwater fever in paralytic subjects artificially inoculated with certain strains of subtertian malaria. A single case of blackwater fever which was fatal has been reported by Fairley (1939) with infection with *P. ovale*. Also numerous cases of blackwater infection in monkeys due to *P. knowlesi* have been described. Wats (1938) reports that in 254 rhesus monkeys infected with *P. knowlesi* and none treated only four escaped death and in the majority haemoglobinuria occurred. Ciuci has also reported a case of blackwater fever in a patient inoculated with *P. knowlesi* following a single dose of atabrin.

It is believed that as a result of the damage done the patient by the malarial attacks there is a tendency on the part of his red cells to haemolysis. A hypothetical autolysin or an anaphylactic sensitizing substance has been suggested to explain the haemolysis.

Malaria apparently is the predisposing cause and the exciting cause may be any of a number of different factors capable of lowering body resistance such as other infections, the occurrence of another malarial attack, the administration of quinine, particularly of the acid salts of quinine in rather large doses, refrigeration as brought about by one's clothes becoming wet and then later subjected to the chilling influence of a sea breeze, to excessive fatigue or dietetic or alcoholic excesses. Quinine administration particularly if associated with refrigeration is the most common exciting factor. Another procedure said to be provocative of blackwater fever is X-ray application to the region of the spleen.

As regards the association of malaria and blackwater fever Stephens in a study of 390 cases of blackwater found that 73 per cent of the cases showed malarial parasites on the day preceding the haemoglobinuria, 47.5 per cent on the day of the attack and 23 per cent on the day following the appearance of the dark urine. Other workers give even higher figures as 95, 70 and 20 per cent. However more recent reports show that in only 50 to 70 per cent of the cases can parasites be demonstrated during the attack. Foy and Kondi (1938) in Greece found that approximately 40 per cent of their cases of blackwater fever were positive for malarial parasites but that the assessment of the parasite rate in blackwater fever



among them although still common among the white Europeans in the same region who neglect these measures

Manson Bahr (1940) points out that blackwater fever occurs not uncommonly in England in individuals of both sexes who have been infected with subtertian malaria in West Africa and other highly malarious countries in whom it is apt to break out after their return on exposure to cold through over indulgence with alcohol or following quinine administration within a period as long as 8 months after their arrival. Such cases are, as a rule acute and the mortality rate has been 50 per cent or even higher

### ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—For a number of years it was suggested that blackwater fever was a disease *sui generis* a completely separate entity. This view was particularly advanced because of the discovery in 1888 of *Babesia* as the cause of redwater fever of cattle. Sir Patrick Manson suggested that some such affection might occur in persons made susceptible by a previous attack of malaria.

Sambon also thought by reason of the clinical resemblance of blackwater to certain haemoglobinuric diseases in cattle (Texas fever), dogs and sheep that such a cause might be operative. These parasites (*Piroplasmata*) of the red cells are easily discernible in the animal infections but have never been seen in blackwater fever.

Schuffner (1918) and Blanchard and Le Frou (1922) believed that they had discovered the specific cause as a spirochaete which resembled *S. icterohaemorrhagica*. However they found bilirubin constantly in the urine which is not found in true blackwater fever. On inoculation of guinea pigs haematuria resulted but not haemoglobinuria. The finding of a spirochaete was never confirmed in other cases of blackwater fever.

Leishman in earlier years noted the presence in the large mononuclear cells of the blood of blackwater patients of certain cell inclusions which he thought to be of chlamydozoal nature and he suggested that these chlamydozoa might be the etiological factor. Such appearances may not only be absent in marked cases of blackwater but have been described in conditions other than blackwater fever.

**Association with Malaria**—Very heavy infections with *P. falciparum* in which as sometimes occurs from 12 to 20 per cent of the erythrocytes are invaded will occasionally show haemoglobinuria. Such cases give support to the old view that haemoglobinuric fever was simply a type of pernicious malaria. Brem has proposed for these cases the designation pernicious malarial fever with haemoglobinuria.

Blackwater fever occurs almost always in those who have resided for considerable periods of time in districts where malignant tertian malaria is very prevalent and intense and who have repeatedly suffered from such malarial attacks. More rarely blackwater fever may be connected with benign tertian or exceptionally with quartan infections. However Stephens formerly could find only 7 such cases in the literature. J. G. Thomson believes that some cases reported as connected with benign tertian malaria are instances of mixed infections with malignant tertian,

coefficient in their cases between the last dose of quinine and the first passage of black urine was so significant as to make it appear that there is more than a causal relationship between the two. Nevertheless in other studies (1937) they point out that though quinine had been taken in the vast majority of cases in others no quinine whatever had been taken. They also observed that people who have been in the hospital and taken the full treatment for malaria with either atebrin or quinine sometimes go down without any warning with blackwater fever their blood and spleens being negative for both parasites and pigment.

**The Quinine Theory**—This idea as to the causation of blackwater fever first originated with Veretas in Greece in 1858. Later Tomaselli supported this view in Italy and more recently it was advocated by Koch. It has been suggested that Koch's insistence on this theory was unfortunate because many persons with severe malaria have refused to take the specific quinine for fear of bringing on haemoglobinuria.

Some maintain that quinine alone even in doses which are capable of producing profound toxic effects such as disturbances of sight and hearing, weak heart and collapse does not cause haemoglobinuria and it has sometimes been stated that quinine base and quinine tannate tend to prevent haemolysis, haemoglobinaemia and haemoglobinuria. It appears definite that blackwater fever sometimes develops without the previous administration of quinine. It has been argued that malaria and not quinine is the chief factor in the etiology of blackwater fever and that this is suggested by the fact that though immense quantities of the specific have been taken for the cure of simple tertian and quartan infections blackwater very rarely supervenes in these and generally only in malignant tertian infections.

Some confusion has resulted from the fact that in especially susceptible individuals the administration of quinine may produce a transient haemoglobinuria but this condition should not be confused with the blackwater fever associated with malarial infection. Manson, Gordon, Thompson, McMillan and others have all reported in earlier years haemoglobinuria following the administration of quinine. In such cases the haemoglobinuria usually develops within an hour after taking the drug. Manson Bahr has pointed out that it has sometimes become necessary to issue a special certificate to such individuals warning medical officers against prescribing quinine for them. Muhlens and Knabe have reported a case of extraordinarily pronounced quinine susceptibility in a young seaman from West Africa whom they were unable to accustom to take the drug. Less than 1 gr. of quinine urethane caused in him an attack of blackwater. On the other hand he showed great tolerance to plasmoquine and total doses of it amounting to 4.75 grm. failed to banish the malarial parasites from his blood.

Connal in Nigeria has reported 24 cases of severe blackwater fever in negroes who had never taken quinine and found that regular quinine takers are less liable to have a fatal attack of the disease than those who have taken the drug in irregular fashion.

can only be determined when a number of factors are taken into consideration such as (1) time and amount of last dose of quinine (2) the magnitude and duration of haemolysis (3) the time that has elapsed between the first passage of black urine and examination of the blood and (4) the parasite index in the general population. These factors they state apply with equal force to spleen puncture findings. Fairley (1937) states that the parasites may be difficult or impossible to find after the first 24 hours.

By examining for increased percentage of large mononuclears or for melaniferous leucocytes in those cases not showing malarial parasites the evidence of malarial etiology sometimes may be additionally increased.

Lamborn (1938) fed 50 *A. gambiae* and 50 *A. funestus* on a European patient at the height of an attack of blackwater fever and subsequently the majority of these fed on two native boys of 10 years of age between the 7th and 32nd day. There were no reactions in either of the boys who were under observation for 4 months. No parasites were found in a search of 17 of the *A. gambiae* and 14 of the *A. funestus* used in the experiment.

Darling during his service in the Panama Canal Zone found malarial parasites at autopsy in every person who died of blackwater fever. However Fairley found that malarial pigment may be unexpectedly scanty in the endothelial cells of the liver and spleen at autopsy. Also it is necessary to point out that a small percentage of cases diagnosed as blackwater fever may not show any evidences of malaria at autopsy and cases are recorded where blackwater has attacked persons who had never had malarial fever. In the latter instance however the haemoglobinuria presumably has another origin and in this connection paroxysmal haemoglobinuria must be considered.

Foy and Koudi (1938) have reported spleen punctures in a series of cases of blackwater fever occurring in Greece. They found that in a certain proportion of cases the spleen might contain small or large amounts of pigment in which there was no trace of infection in the peripheral blood. However, in some of their cases when the peripheral blood harbored numerous parasites the spleen contained only most meagre traces of pigment and these results applied to infections with both *P. vivax* and *P. falciparum*. They found further that there was little or no evidence of heavy malarial infection just prior to the onset of blackwater fever in the great majority of the cases and that if the presence of pigment in the spleen might be taken as evidence of recent schizogony then very few cases of blackwater fever can be said to be suffering from active malaria at the time of the onset of the haemoglobinuria. The rate at which the spleen disposes of pigment seemed to be extremely variable and not to be dependent upon the time or the amount of quinine taken prior to the onset of the blackwater fever. They thought that the reticulo-endothelial system appeared to play an important role in the destruction rate of the pigment. They point out although quinine is by no means an essential prerequisite of blackwater fever that the correlation

coefficient in their cases between the last dose of quinine and the first passage of black urine was so significant as to make it appear that there is more than a causal relationship between the two. Nevertheless in other studies (1937) they point out that though quinine had been taken in the vast majority of cases in others no quinine whatever had been taken. They also observed that people who have been in the hospital and taken the full treatment for malaria with either atebrin or quinine sometimes go down without any warning with blackwater fever, their blood and spleens being negative for both parasites and pigment.

**The Quinine Theory**—This idea as to the causation of blackwater fever first originated with Veretas in Greece in 1858. Later Tomaselli supported this view in Italy and more recently it was advocated by Koch. It has been suggested that Koch's insistence on this theory was unfortunate because many persons with severe malaria have refused to take the specific quinine for fear of bringing on haemoglobinuria.

Some maintain that quinine alone even in doses which are capable of producing profound toxic effects such as disturbances of sight and hearing, weak heart and collapse does not cause haemoglobinuria and it has sometimes been stated that quinine base and quinine tannate tend to prevent haemolysis, haemoglobinaemia and haemoglobinuria. It appears definite that blackwater fever sometimes develops without the previous administration of quinine. It has been argued that malaria and not quinine is the chief factor in the etiology of blackwater fever and that this is suggested by the fact that though immense quantities of the specific have been taken for the cure of simple tertian and quartan infections blackwater very rarely supervenes in these and generally only in malignant tertian infections.

Some confusion has resulted from the fact that in especially susceptible individuals the administration of quinine may produce a transient haemoglobinuria but this condition should not be confused with the blackwater fever associated with malarial infection. Manson, Gordon, Thompson, McMillan and others have all reported in earlier years haemoglobinuria following the administration of quinine. In such cases the haemoglobinuria usually develops within an hour after taking the drug. Manson Bahr has pointed out that it has sometimes become necessary to issue a special certificate to such individuals warning medical officers against prescribing quinine for them. Muhlens and Knabe have reported a case of extraordinarily pronounced quinine susceptibility in a young seaman from West Africa whom they were unable to accustom to take the drug. Less than 1 gr. of quinine urethane caused in him an attack of blackwater. On the other hand he showed great tolerance to plasmoquine and total doses of it amounting to 4.75 grm. failed to banish the malarial parasites from his blood.

Connal in Nigeria has reported 24 cases of severe blackwater fever in negroes who had never taken quinine and found that regular quinine takers are less liable to have a fatal attack of the disease than those who have taken the drug in irregular fashion.

can only be determined when a number of factors are taken into consideration such as (1) time and amount of last dose of quinine (2) the magnitude and duration of haemolysis (3) the time that has elapsed between the first passage of black urine and examination of the blood, and (4) the parasite index in the general population. These factors they state apply with equal force to spleen puncture findings. Fairley (1937) states that the parasites may be difficult or impossible to find after the first 24 hours.

By examining for increased percentage of large mononuclears or for melaniferous leucocytes in those cases not showing malarial parasites the evidence of malarial etiology sometimes may be additionally increased.

Lamborn (1938) fed 50 *A. gambiae* and 50 *A. funestus* on a European patient at the height of an attack of blackwater fever, and subsequently the majority of these fed on two native boys of 10 years of age between the 7th and 32nd day. There were no reactions in either of the boys who were under observation for 4 months. No parasites were found in a search of 17 of the *A. gambiae* and 14 of the *A. funestus* used in the experiment.

Darling during his service in the Panama Canal Zone, found malarial parasites at autopsy in every person who died of blackwater fever. However Fairley found that malarial pigment may be unexpectedly scanty in the endothelial cells of the liver and spleen at autopsy. Also it is necessary to point out that a small percentage of cases diagnosed as blackwater fever may not show any evidences of malaria at autopsy and cases are recorded where blackwater has attacked persons who had never had malarial fever. In the latter instance however the haemo-globinuria presumably has another origin and in this connection paroxysmal haemoglobinuria must be considered.

Foy and Koudi (1938) have reported spleen punctures in a series of cases of blackwater fever occurring in Greece. They found that in a certain proportion of cases the spleen might contain small or large amounts of pigment in which there was no trace of infection in the peripheral blood. However, in some of their cases when the peripheral blood harbored numerous parasites the spleen contained only most meagre traces of pigment and these results applied to infections with both *P. vivax* and *P. falciparum*. They found further that there was little or no evidence of heavy malarial infection just prior to the onset of blackwater fever in the great majority of the cases and that if the presence of pigment in the spleen might be taken as evidence of recent schizogony then very few cases of blackwater fever can be said to be suffering from active malaria at the time of the onset of the haemoglobinuria. The rate at which the spleen disposes of pigment seemed to be extremely variable and not to be dependent upon the time or the amount of quinine taken prior to the onset of the blackwater fever. They thought that the reticulo-endothelial system appeared to play an important role in the destruction rate of the pigment. They point out although quinine is by no means an essential prerequisite of blackwater fever that the correlation

that a haemolysin may be free in the blood stream or bound in certain cells but it is not clear what suddenly frees it or precipitates its action

Dudgeon thought that haemolytic substances were present in the tissues and urine. However the efforts to isolate such a haemolysin have not been successful and the mechanism of the production of haemoglobinuria is still only partially understood. The predisposing causes of an attack especially quinine have already been referred to. It is clear that an acute haemolysis of the red blood corpuscles occurs and that this liberates haemoglobin into the blood stream and thus haemoglobin being excreted by the kidney in turn results in haemoglobinuria. One frequently finds cylindrical plugs of highly albuminous haemoglobin containing coagula in the tubules of the kidneys. Following this haemolysis in the blood bilirubin and pseudo methaemoglobin may also appear in increased amounts in the blood stream and account for the characteristic icterus of the disease. Nocht believes there are 3 factors which produce the condition (1) a haemolytic factor due to the reduction of cholesterol in the blood (2) a loss in its protective effect and (3) the administration of the toxic quinine.

Krishnan (1938) who has carried out biological studies of the blood in cases of blackwater fever and in malarial haemoglobinuria finds that the total cholesterol figures were lower than the normal values while the figures obtained for organic phosphorus were on the whole higher than normal. These changes indicate that there is not only a derangement of the fat metabolism but also prior to haemolysis there is an upset in the lecithin free-cholesterol ratio. The nature of the biochemical changes described in monkeys prior to the onset of haemoglobinuria suggested that these changes may be the result of a profound injury to the liver. Histological examinations of the liver and adrenals confirmed this hypothesis.

Ross in Rhodesia employed the van den Bergh reaction for bilirubin in 17 cases of blackwater fever and reported an indirect reaction varying from 5.2 to 59.0 units. (One unit corresponds to 0.5 mg per 100 cc.) Kingsbury also employing the van den Bergh reaction found that in 90 per cent of the cases of uncomplicated subtertian malaria the serum bilirubin is above the normal figure which is never over 0.5 mg per 100 cc and consequently urobilin derived from the serum bilirubin is found in pathological amounts in the urine of these cases. In blackwater fever and in severe subtertian malaria this excessive bile leads to bilious vomiting. Normally the bilirubin is excreted by the liver resulting in an increased flow of bile.

Kingsbury believes that usually in a severe case of subtertian malaria there is some haemoglobinaemia or the liberation of free haemoglobin into the blood serum but that this is immediately dealt with by the reticulo endothelial system while in blackwater fever the liberation of haemoglobin is so extensive and so rapid that the renal threshold for free haemoglobin is broken down and the pigment appears in the urine. Even when haemoglobinuria occurs most of the haemoglobin is broken up by the usual mechanism and only a relatively small portion (not more than one third) is excreted in the urine. Yorke, Murgatroyd and Owen have also demonstrated that where there is an intravascular haemolysis of sufficient intensity to exceed the renal threshold for haemoglobin the blood pigment appears in the urine but that even in the most severe cases of blackwater fever never more than 10 per cent of extra corpuscular

Duren (1938) reports that the medical corps in the Congo is almost unanimous in looking upon chronic *falciparum* malaria as the essential cause of haemoglobinuria the direct exciting cause being chills, privation overwork or quinine. Those who take prophylactic quinine regularly are very rarely attacked and there is generally in the cases of blackwater fever a history of a dose of quinine taken during an attack of fever by a person who does not take the drug prophylactically. Recently Nocht, Stephens, Christophers and other prominent malarialogists have stressed again the malarial basis of blackwater fever and the importance of quinine as a precipitating factor in the haemolysis.

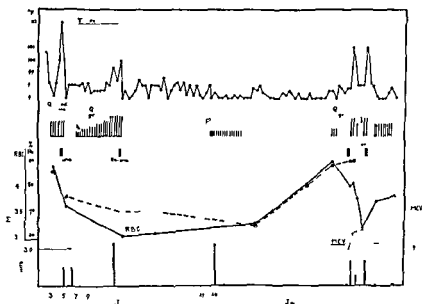


Chart of data in recurrent attacks of blackwater fever induced by quinine (Case of Doctors N. Hamilton Fairley and F. Murgatroyd. Courtesy Roy Soc Trop Med & Hyg. London)

Fairley and Murgatroyd (1940) have reported a case with 4 recurrent attacks of mild blackwater fever induced in each instance by quinine. Such attacks could be produced while the malarial fever persisted. However, for several months after apparent cure quinine therapy entirely failed to induce haemoglobinuria. Their evidence suggested that the lytic agent acts directly on the circulating corpuscles and suggests a lytic enzyme or biological haemolysin may be implicated. As noted above blackwater fever has been produced in patients by inoculating them with strains of subtertian malaria and a similar disease has been produced in monkeys by inoculating them with *Plasmodium knowlesi*.

**Mechanism of Haemolysis**—A scientific explanation of the mechanism by which haemolysis is brought about is not yet possible. It is conceivable

that a haemolysis may be free in the blood stream or bound in certain cells but it is not clear what suddenly frees it or precipitates its action

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haemoglobin is excreted by this route. They believe the remaining 90 per cent of pigment has to be dealt with by the liver and reticulo-endothelium.

Tonick has carried out many experiments to see what the concentration of haemoglobin in the blood must be before it is excreted by the kidneys into the urine. The results showed considerable individual variation but in all the positive cases the serum appeared quite pink in color. According to Pierce the blood must contain at one time more than 0.06 gm of free haemoglobin per kilo of body weight in order to produce haemoglobinuria in man.

Plehn suggested the idea that there existed a supersensitiveness of the patient to the protein of the malarial parasite from repeated febrile attacks and that when this supersensitiveness was attained to a sufficiently high degree further destruction of quite a small number of parasites might set free more such protein and precipitate the attack of blackwater fever. Hence he argued that the haemolysis takes place in the kidneys causing a great disturbance in their function. The kidneys being no longer able to excrete bilirubin this becomes present in the blood in blackwater fever cases. However Plehn's hypothesis that the primary lesions lay in the kidney and that blood escaped directly into the urine where it was laked owing to the absence of salts and urea has been discarded.

DeLangen (1936) in agreement with Plehn but in contrast with other recent work believes also that the haemolysis does not take place in the blood stream. Since there is very little free haemoglobin in the circulation and since the kidneys throw out such tremendous amounts of it he thinks that the erythrocytes must be destroyed in the kidneys. The appearance of urobilin is explained on the assumption that the erythrocytes are also broken down in the liver and spleen and that the liberated haemoglobin is converted directly into urobilin though it is conceived possible that a portion of the bilirubin may be formed extra hepatically in the kidneys for instance.

With reference to Plehn's theory of supersensitiveness Fernán Gúnez (1936) has assembled evidence to support the theory that the attack is an acute allergic reaction to the malarial protein. He prepared an antigen by concentrating parasites by Bass and John's method and adding 0.4 per cent formalin and tested hypersensitiveness by intracutaneous injections of 0.2 cc. In a group of 410 individuals of white or mixed blood who had lived in an endemic area in Colombia for 6 months or more and who had had aestivo-autumnal malaria he obtained 16 positive reactions (a local area of inflammation appearing within 12 hours). After these hypersensitive cases had been sent to a non-malarial district no further cases were observed in the community.

MacGilchrist earlier advanced the idea that blackwater fever was brought about by a state of acidosis in an individual with a damaged liver plus malaria and the administration of acid salts of quinine. He thought that one can safely give quinine when alkalis are being given and that quinine base is protective against haemolysis.

Fairley and Bromfield (1934) after extensive studies have produced evidence suggesting that the haemolytic agent in blackwater fever arises from some metaplastic breakdown in malignant malaria which is precipitated by the administration of quinine or plasmochin. The corpuscle becomes first lysed and second the liberated oxyhaemoglobin is converted into methaemoglobin and other pigments. Apparently this haemolytic agent may be present in variable quantity in different cases and at different stages of the disease. The observations of MacGilchrist that an acidosis is developed has been confirmed. The plasma bicarbonate shows some times a definite increase and there may be a definite lowering of the alkali

reserve associated with urea retention. While acidosis is apparently usually present. Ross in the study of 4 cases of the disease in Rhodesia found no evidence that an acidosis existed.

With reference to the further development of the disease and the changes in the kidneys Baker and Dodds (1925) found that in rabbits injected with haemoglobin the urine with a pH of 6.0 or less and a concentration of NaCl exceeding 1 per cent in the tubules favored the formation of methaemoglobin from oxyhaemoglobin and ultimately of acid haematin which they believed constituted the precipitate blocking the lumina of the tubules. When the urine was made alkaline the oxyhaemoglobin remained unchanged and was passed as such without renal damage resulting. Ross (1932) generally confirmed this experimental work in blackwater fever but recorded certain anomalies such as the presence of oxyhaemoglobin in the urine with a pH of 5.5 and the presence of both methaemoglobin and oxyhaemoglobin in urine with a pH of 6.8. He found the amount of urea in the blood invariably raised especially in cases with threatened suppression of urine.

Yorke has also shown that the blood urea commonly rises in uncomplicated cases of blackwater fever to 65 mg per cent on the fourth day of the disease. Fairley in severe toxic cases also observed a tendency for a rise in blood urea to take place when percentages between 27 and 52 mg per cent were obtained.

**Plasma Pigments**—Yorke, Murgatroyd and Owen (1930) and Fairley and Bromfield (1933) by an improved technique for quantitative estimation concluded that malaria is unaccompanied by haemoglobinaemia though they thought it might be found in cases of primary hyperinfection producing severe anaemia and intense jaundice. Voigt (1938) in the course of work upon the etiology of blackwater fever has examined a number of cases of malignant tertian malaria with respect to the haemoglobin content of the true plasma. In the 17 cases of malignant tertian malaria studied in all of which *P. falciparum* was found in the blood 9 showed no haemoglobin in the plasma, 4 showed traces and the remaining 4 from 22 to 60 mg per 100 cc. In no case was methaemoglobinaemia observed. In 25 control cases (with no malaria) in only 9 was oxyhaemoglobinaemia detected in amounts between 5 and 19 mg per 100 cc of plasma and none had methaemoglobinaemia.

These recent observations are in agreement with the opinion that there is little or no evidence that demonstrable haemoglobinaemia occurs in the ordinary malarial paroxysm. Nevertheless haemoglobinaemia is present in blackwater fever and this either directly or indirectly is the source of the different blood pigments which appear in the urine in this disease.

Regarding the haemoglobinaemia in blackwater fever Fairley and Bromfield (1937) in their quantitative studies in a large series of cases in London and Macedonia invariably found toxic haemoglobin in the plasma in significant amounts provided specimens were collected at a time when haemoglobinauria was still present. The maximum quantity observed was 522 mg per 100 cc or 3.77 per cent (Haldane's scale). In view of this massive blood destruction Yorke commented upon the

small amount of blood pigment actually demonstrable in the plasma and suggested that the relatively low plasma haemoglobin values in black water fever might be due to the haemolysis taking place in the sinuses of the spleen and liver the haemoglobin reaching the blood stream more slowly than in *Babesia* infection of dogs where as much as 12 per cent of extra corpuscular pigment had been observed Yorke has also raised the question whether the hypertrophied reticulo endothelial cell system may not play a roll in the haemolysis

In addition to the presence of oxyhaemoglobin methaemoglobin has been recorded in blackwater fever by various observers However, in 1934 Fairley and Bromfield reported a new pigment closely allied to methaemoglobin in the plasma of a patient with blackwater fever over a period of 10 days and more recently (1937) they found that this pigment was invariably present in all the more severe cases of blackwater fever studied in Macedonia It is a brownish pigment resembling methaemoglobin spectroscopically and not reduced by Stokes's reagent or ammonium sulphide though it contains a trivalent iron molecule While the spectrum has the general appearance of methaemoglobin, the bands are shifted It is a non threshold substance and hence does not appear in the urine

They showed that this pigment had been erroneously recorded as methaemoglobin by all previous workers on the subject and named it pseudo methaemoglobin Subsequently it was termed methaemalbumin Quantitative observation based on the extinction coefficient of the two pigments indicates that the maximum concentration of pseudo methaemoglobin is attained later than that of oxyhaemoglobin while in fatal cases its concentration often progressively rises until death whereas that of oxyhaemoglobin falls Graphs of such cases indicate that the new pigment originates from extra corpuscular haemoglobin Though on direct spectroscopic examination its spectrum closely resembles that of methaemoglobin the new pigment can readily be distinguished by examination on the Hartridge reversion spectroscope set against artificially produced methaemoglobin when the alpha band in the red is found to be displaced nearer the blue end of the spectrum The alpha band of methaemoglobin is 6300 Å whereas that of pseudohaemoglobin approximates 6230 Å In addition the behavior of the two pigments to certain chemical reagents is quite different Pseudo methaemoglobin cannot function as a respiratory pigment It is never found within the corpuscles nor does it appear in the urine in demonstrable quantities The view that it is derived from oxyhaemoglobin only after its liberation from red cells is borne out by experimental evidence

Believing that this pigment was peculiar to blackwater fever, Fairley and Bromfield postulated that the plasma of this disease must contain some peculiar substances of metabolic origin responsible for its formation from extra corpuscular haemoglobin The results of their experiments to test this hypothesis have indicated that the plasma has the power of producing pseudo methaemoglobin directly or indirectly from extra

corpuscular haemoglobin and suggest that in severe intravascular haemolysis from any cause pseudomethaemoglobin would be produced.

The subsequent stages of katabolism of pseudo methaemoglobin are unknown but presumably it is adsorbed from the circulation by the reticulo endothelial cells following which the haemoiety is converted into the iron containing pigment haemosiderin and the iron free pigment haemobilirubin.

Foy and Kandi in Greece have confirmed the occurrence of pseudo methaemoglobin in the plasma and serum of blackwater fever. Methaemoglobin was found in the urine but never in the serum. They also found methaemoglobin but not pseudomethaemoglobin in the blood of a patient with cyanosis resulting from plasmoquine. The methaemoglobin was intracorpuscular and never appeared in the serum which is in contrast with the pseudomethaemoglobin of blackwater fever which is always free in the serum.

Hewitt (1938) who has investigated the structure of methaemalbumin (pseudo-methaemoglobin) found it to be a peculiar haemoglobin derivative with a normal prosthetic group but the globin portion of the molecule modified. It could be produced artificially by the addition of the serum to alkaline haematin. However it was not certainly determined whether the pigment is merely a combination of haematin and serum albumin. He has found that several proteins including crystalbumin, globoglycoid and seroglycoid are contained in the albumin fraction and on adding haematin to solutions of these different proteins Fairley and Bromfield (1939) found pseudomethaemoglobin was immediately found in the case of crystalbumin but not of the other two. It seems clear that the haemolytic agent may be present in variable quantity in different cases and at different stages of the same case.

**Hyper bilirubinaemia**—The presence of bile pigment in the blood plasma in blackwater fever has long been recognized. Plehn, Christophers and Bentley noted that icterus was associated with intense yellow coloring of the serum. The latter author also noted the absence of bile salts in the urine.

Attention has been called to the investigation of the van den Bergh reaction in cases of blackwater fever and to the fact that Ross, Kingsbury, Whitmore and Rowe have obtained indirect reactions.

Fairley and Bromfield in a series of 20 severe cases found that the indirect reaction in 8 fatal cases varied from 5.0 to 80.5 units and in the 12 cases that recovered from 5.0 to 6.0 units (1 unit = 0.5/mg per 100 cc). The average maximal readings for the total series based on 96 estimations was 1.4 units of 0.7 mg per 100 cc. Hyperbilirubinaemia occurred in all cases and a considerable degree of jaundice. A hyperbilirubinaemia was compatible with recovery.

**Urinary Pigments**—The pigments of especial interest of the urine are oxyhaemoglobin, methaemoglobin, urobilin and a brown pigment demonstrable in the centrifuge deposit which is generally regarded as acid haematin and responsible for the blockage of the renal tubules. This last pigment however has not the solubility of artificially produced

acid haematin and is difficult to investigate spectroscopically owing to its insolubility. The quantity of oxyhaemoglobin present in the urine varies considerably in different cases. It is presumed to originate from extra corpuscular circulating haemoglobin. The extra-corpuscular haemoglobin is filtered through the glomerulus whenever its concentration exceeds the renal threshold its molecular rate of 68 000 being apparently of an order which would just permit glomerular filtration. However Yorke (1937) believes that haemoglobin is secreted by the epithelial cells of the convoluted tubules which are damaged in the process and undergo degenerative changes.

Methaemoglobin is responsible for the black discoloration of the urine in blackwater fever and its incidence varies with the reaction of the urine, the time the urine has been retained in the bladder and the period intervening between its collection and examination. Contamination and ammoniacal fermentation may lead to its disappearance altogether through the formation of alkaline methaemoglobin which does not present an alpha band in the red portion of the spectrum. However on acidification with acetic acid methaemoglobin reappears.

Fairley in most of his recent Macedonian series found most of the blood pigment present in the form of methaemoglobin. Values of from 9 to 905 mg per 100 cc were found and when anuria was present even higher concentrations were obtained. On the other hand in the transient haemoglobinurias especially those of children this pigment may never be demonstrated.

The origin of the methaemoglobin in the urine has been disputed. Fairley believes that it is formed from oxyhaemoglobin in the renal tubules and bladder rather than from the circulating blood since the discovery that methaemoglobinæmia so called in blackwater fever is really a pseudo methaemoglobinæmia. Pseudo methaemoglobin (methaemalbumin) has not been found in the urine. Yuile (1942) has given a critical survey of the further experimental findings relating to haemoglobinuria in an attempt to reach a clearer understanding of these factors.

**Paroxysmal Haemoglobinuria**—It is important to differentiate the blood changes in blackwater fever from those present in paroxysmal haemoglobinuria. It may be pointed out that the chief characters of paroxysmal nocturnal haemoglobinuria of the Marchiafava type are an anaemia of the chronic haemolytic type associated with jaundice and persistent haemoglobinæmia. The accompanying haemoglobinuria occurs only or is most severe at night. Ham (1937) and others have found that the red blood cells of these patients both *in vitro* and *in vivo* are abnormally susceptible to haemolysis in plasma of increased acidity within the physiologic range of pH variation. Dacie, Israels and Wilkinson (1938) also found in the study of a case of this disease that autohaemolysis could be demonstrated *in vitro* and was shown to be dependent upon the pH of the system. The optimum pH for lysis was approximately 7.0-7.2 and it could not be produced at a pH greater than 7.8. Autohaemolysis of whole blood will occur at 37°C without preliminary chilling cold being the precipitating factor of the autohaemolysis in another form of paroxysmal haemoglobinuria (Donath Landsteiner reaction). It is suggested that the patient's red cells are sensitive to a potential lysin present in normal serum. The interaction between the patient's red cells and this

lysin is regarded as probably responsible for the clinical picture of the severe haemolytic anaemia with nocturnal haemoglobinuria Ham (1937) suggests that the slight decrease in the pH of the peripheral blood during sleep may not entirely reflect the acidity of certain other regions of the body. He thought in nocturnal haemoglobinuria the destruction of the red blood corpuscles occurred within the splenic pulp.

Yorke has recently devised a test which is said to be valuable for the purpose of differentiating blackwater fever from paroxysmal haemoglobinuria. Some blood is withdrawn and the serum separated. It is then cooled to the freezing point and subsequently warmed to 37°C. In paroxysmal haemoglobinuria the serum then shows an active haemolytic action of the patient's red cells. On the contrary this has not been found to occur in blackwater fever cases. In the serum of paroxysmal haemoglobinuria the immune body is greatly in excess of the complement whereas in blackwater fever the reverse is true. Recent investigations by Donath and Landsteiner suggest that in paroxysmal haemoglobinuria (precipitated by cold) haemolysis takes place in the peripheral blood. The suggestion of other investigators that in blackwater fever haemolysis may occur particularly in the kidneys has already been referred to.

Fairley and Bromfield (1939) in investigating the plasma pigments of cases of paroxysmal nocturnal haemoglobinuria on examination of the blood with the Hartridge reversion spectroscopie have found the alpha band in the red portion of the spectrum to be colinear with the alpha band of pseudo methaemoglobin (6230 Å) which they had previously demonstrated in the plasma from cases of blackwater fever and to be quite distinct from that of methaemoglobin (6300 Å). Further while Stokes reagent ammonium sulphide (10 per cent) sodium fluoride hydrazine hydrate (50 per cent) and hydrogen peroxide (10 vols) dispersed the alpha band of methemoglobin immediately the alpha band of pseudo methaemoglobin was found to persist in the presence of the first 3 reagents and only gradually to be dispersed by the last 2. Fairley concludes that there can be no reasonable doubt therefore that the pigment in question is pseudo methaemoglobin and not methaemoglobin as previously reported by a number of other investigators in studying nocturnal haemoglobinuria. Fairley has more recently suggested the name of methaemalbumin for this substance.

In connection with the study of the etiology of blackwater fever the important investigations of Dameshek 1937-39 and Dameshek and Schwartz 1939 upon haemolysins as the cause of certain haemolytic anaemias should be carefully considered. They found an active lysin in the serum of three cases of acute haemolytic anaemia. This lysin possessed all the criteria of an immune body being inactivated by heat and reactivated on the addition of complement. Since the most fulminating case of the series showed very marked microspherocytosis with greatly increased red cell fragility and since this abnormality disappeared as the patient improved after splenectomy the assumption was drawn

that the spherocytosis, and the increased fragility might be due to the action of haemolysin. That this was the case was demonstrated by experimental studies in which haemolysins for guinea pig red cells were produced in rabbits and then injected into guinea pigs. Acute fulminating haemoglobinuria, acute haemolytic anaemia, and sub acute haemolytic anaemia were produced at will depending on the single factor of dosage.

**Epidemiology**—There seems to be a general opinion that when malaria is kept in check by antimalarial measures blackwater fever usually becomes mild in character and may become rare or even extinct in a community. It is often in those who are careless about these prophylactic measures or who expose themselves to depressing influences as cold wet excessive fatigue or alcoholic debauches, that blackwater is more likely to develop.

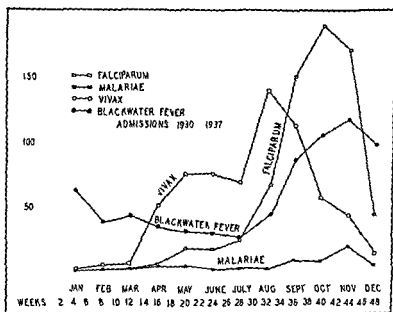


FIG. 31.—Monthly distribution of *malax falciparum* and *malaxiae* compared with blackwater fever admission (partly from Balfour). (From Poy & Kond courtesy of Roy Soc Trop Med & Hyg London.)

Over exertion leading to fatigue and chilling seems to be one of the most common exciting factors. Those in bad health from disease or lack of proper diet also seem more susceptible. A peculiar feature regarding the disease has been reported namely that it may be absent in a district for a number of years and then assume almost epidemic proportions. It has been suggested that such outbreaks may depend upon the number of new arrivals in the endemic region. Thus it was noted that Chinese laborers who were imported for work on the Congo railway were especially attacked. At one time race was considered to be an important factor as in Africa, Europeans, Indians and Chinese were

especially attacked while the Negroes were apparently immune. Plehn however observed serious outbreaks of blackwater fever among the Negroes from the Cameroon Mountains when they came to the coast from the interior. Manson Bahr has called attention to the not infrequent occurrence of blackwater fever in apparently healthy persons who have arrived in England and who have come from malarious regions. He explains these cases as probably due to the fact that the subtertian malarial infection is lying latent until aroused into activity by exposure to cold or some other depressing influence.

A rough seasonal incidence of the disease has been noted. It is especially frequent in late summer and in the autumn. On the West Coast of Africa it is reported most prevalent at the close of the rainy season in August and September but in the highlands and in central Africa it usually reaches its height during the wettest months May to August when the lowest temperatures are reached. In Greece the bulk of the blackwater fever cases occur in the period from November to February which is roughly 2 months after the peak for clinical malaria in that region. During the Great War cases of blackwater fever occurred among the troops in Salonika and in Palestine almost only during the cold or winter months. In Macedonia it reached its height in September–November.

Europeans are usually exempt from attacks during their first year in endemic tropical areas. Dudgeon obtained a malarial history in every one of a hundred cases observed by him in the Balkans.

#### PATHOLOGY AND MORBID ANATOMY

As a result of the excessive destruction of red cells the liver cannot convert the great amount of haemoglobin released into bile pigment so that haemoglobinaemia and haemoglobinuria result. Severe red cell destruction as by toluene diamine in a rabbit may not be followed by haemoglobinuria. In such a case phagocytosis of red cells may be the explanation. It has been estimated by Ponfick that if one sixth of the red cells are destroyed the liver is unable to dispose of the liberated haemoglobin and haemoglobinuria results. A formerly damaged liver would be less competent. Discussions as to autolysins and complement content of serum have arisen. Knowledge on these points is deficient.

Dudgeon has reported active haemolysins in the tissues and urine of blackwater fever cases which he was unable to find in other conditions including malaria. Other workers have failed to find haemolysins. He obtained no evidence of increased fragility of the red cells and no evidence of auto haemolysis. Bile pigment in the plasma occurred in most of the cases which ended fatally.

As a rule the pathological changes observed in blackwater fever are those associated with malaria. They include renal congestion congestive enlargement of the spleen and liver and distension of the gall bladder with thick blackish bile and usually haemosiderosis of the spleen liver and kidneys. Also sometimes hypertrophy of the bone marrow extending



into the long bones Whipple and others have pointed out that there may be congestion of the kidneys with purple colored pyramids The kidneys often show cellular desquamation and granular disorganization in the convoluted tubules Granular eosinophile material forming casts may block the straight and collecting tubules In the spleen the Malpighian bodies are often prominent and sharply outlined Very striking are the necroses of the Malpighian corpuscles of the spleen and focal necroses of the liver which may occur Whipple considers that this speaks for a powerful circulating toxin in blackwater fever which is not present in malaria The spleen sinuses are sometimes distended and there may be autoagglutination of red cells together with active phagocytic action of the endothelial cells In Dudgeon's opinion the presence of the Prussian blue reaction of haemosiderin in the liver spleen and renal epithelium is the most characteristic feature of blackwater fever Fairley also emphasizes the greatly increased amount of this pigment in the parenchyma cells of the liver, spleen and kidneys However it may be mentioned that this pigment may occur in any other disease where blood destruction is considerable It contains 17 per cent of iron and is probably the main form in which iron is normally conserved in the body for reutilization in the formation of fresh haemoglobin by the bone marrow

The liver cells in the area of the central veins may show the most marked destruction either cloudy swelling or necrosis The gall bladder is almost always distended with bile which is viscid in consistency and often black in color There have been few quantitative observations made on the bilirubin content of blackwater fever bile, but Fairley and Bromfield in 3 fatal cases found 1.9, 1.3, and 2.45 per cent or from 4 to 7 times the concentration usually found in normal cases Stercobilinogen and stercobilin which are formed directly from cholybilirubin by bacteria are also markedly increased in the faeces and it is believed that it is absorption of stercobilinogen in excess together with the disturbed function of the overtaxed liver which produces the urobilinuria, so characteristic in this disease The myocardium often shows fatty changes and the fat lipoid content of the adrenal may be reduced Krishnan (1937) has noted especially degenerative changes in the adrenal cortex in monkeys dying of *P. knowlesi* infection The urine shows a reddish to black color and has a heavy sediment made up of granular debris hyalin and haemoglobin tube casts with haematoidin crystals and only rarely a red cell Haematuria is not present With the spectroscope the absorption bands of methaemoglobin as well as of oxyhaemoglobin, may usually be observed Urobilin and albumin are usually present in large quantities

#### SYMPTOMATOLOGY

**Onset of Symptoms**—The onset of blackwater fever is usually sudden Any incubation period is very indefinite Very few or none of the patients who suffer with blackwater fever complain of any serious illness or fever just before they pass black urine for the first time Foy has found the

vast majority 70 to 80 per cent say that they felt a little out of sorts and took a large or small dose of quinine within a few hours before passing black urine. In the majority of his cases it seems clear that they were not suffering from the acute effects of malaria before they became ill with blackwater fever.

**A Typical Case**—In a person who has lived in an intensely malarious region for 1 or 2 years or even long after he has left such districts and who has had several malarial attacks there comes on what is considered as another malarial chill which may or may not be definitely connected with some resistance lowering influence such as exposure to tropical sun or rain or indulgence in dietary or other excesses or following in 1 to 6 hours the accustomed dose of quinine. This chill however is more prostrating than the formerly experienced and upon passing his urine the patient notes its reddish to black coffee color and may make the diagnosis of blackwater fever himself. The attack usually comes on suddenly with a very severe chill marked prostration and pain over the region of the kidneys. The temperature in a typical case rapidly goes up to 104° to 105° F. Rather profuse sweating accompanies the fall of the fever and the patient is markedly debilitated after the subsidence of the fever. There may be a recurrence of the paroxysm the following day. The fever course however may be more or less continuous or remittent. In other words it tends to be irregular and atypical.

Nausea and bilious vomiting due to excess of bile come on early with epigastric distress. Almost as pathognomonic as the haemoglobinuria is the early and intense jaundice. This comes on within a few hours or almost simultaneously with the haemoglobinuria and usually lasts for 2 or 3 days after the haemoglobinuria and fever have ceased. Itching of the skin during the jaundice is not noticeable. The spleen and liver are often enlarged and tender. Albuminuria comes on with the haemoglobinuria and from 0.1 to 0.4 of 1 per cent of albumin by weight may be present.

The pulse usually is rapid 110 to 120 from the first but soon becomes feeble and of low tension. In severe cases the very rapid almost thready pulse with pallor and cold extremities may resemble a severe haemorrhage. Epistaxis is not uncommon. A very unfavorable symptom seems to be hiccough. A frequent cause of death and one against which we chiefly direct our therapeutic measures is anuria with subsequent uraemic symptoms coma and convulsions. At times a nephritis may develop in the course of a blackwater attack and the case subsequently run as one of severe nephritis.

Very striking is the rapidly developing anaemia some cases showing a diminution of two million red cells per cmm in 24 hours. The mind is usually clear throughout an attack the patient showing restlessness and marked anxiety. In mild cases the fever course and haemoglobinuria is over within 24 hours leaving the patient far more prostrated than would a malarial paroxysm. In severe cases however the fever runs a remittent course over several days with more marked haemoglobinuria and jaun

dice There may be cases which only show haemoglobinuria These apyretic cases have been considered by some as quinine haemoglobinuria

### SYMPTOMS IN DETAIL

*Fever Course*—This resembles that of a malarial paroxysm and may be intermittent in character or last several days as a remittent fever The rigor which accompanies the febrile rise is intense At times successive paroxysms of chill fever and haemoglobinuria suggest a relapsing type of fever

*The Liver and Spleen*—As a result of the marked blood destruction the liver is unable to dispose of the haemoglobin outpouring and icterus which usually comes on in a few hours and is intense is almost constant together with epigastric distress, bilious vomiting and tenderness and slight enlargement of the liver The spleen is also usually somewhat enlarged and quite tender There are cases of a subacute type, where jaundice and dark colored urine are pronounced but with only moderate fever and but slight gastric and nervous symptoms

*The Circulatory System*—At first the pulse is rapid with high tension but soon it becomes weak compressible and of low tension In severe cases it may have a rate of 150 or more or even become thready

*The Genito urinary System*—The dark colored urine is pathognomonic of the disease and gives it its name The reddish to almost black color is due to haemoglobin or methaemoglobin and not to bile However bile pigments appear in the urine There is but rarely a red cell to be found in the granular debris with occasional haematodin crystals which forms the urinary sediment hence a condition of haemoglobinuria and not of haematuria exists

The urine resists decomposition for a long time Albumin is present in large amount and comes on with the onset of haemoglobinuria Casts are abundant and urobilinuria is marked As a result of the blocking up of the renal tubules with haemoglobin casts pain over the loins and anuria may occur There may be vesical tenesmus

Cases of pregnancy with blackwater fever have been reported by Stephens (1937), Thompson (1939) Thomas and Millen (1939), and Foy and Kondi (1941) Only in the case of Foy and Kondi was the baby available for necropsy The mother was given 1 gm of quinine bishydrochloride by intramuscular injection on the sixth of November Twenty nine hours after the quinine injection she first passed black urine and continued to do so for several days On the ninth of November she gave birth to a 7 months baby which died 3 hours after birth The placenta was intact and appeared normal The blood from it contained innumerable *fa'ciparum* schizonts No malarial parasites or pigment were found in the infant's blood or spleen Although the mother's blood contained methaemalbumin there was no trace of it in the child's blood and it seemed evident that this pigment does not pass from the mother across the placenta to the child It has already been noted that this pigment does not normally pass across the kidney to be excreted in the urine

*The Blood*—Cases have been reported where the red cells have been destroyed so rapidly within 24 hours that the count has fallen from five million to two million per cmm thus producing the rapid and marked anaemia that characterizes the disease. Manson Bahr reports cases in which the red blood cells may number only one million and in which there is an excessive leukopenia with haemoglobin not over 10 per cent and an appearance of microcytes and megaloblasts in the blood.

The blood is usually thin and the serum tinged. The degenerative changes of the red cells are not as commonly seen as one would expect but this is probably due to the fact that degenerated cells are first destroyed in the excessive haemolysis. Anisocytosis and poikilocytosis may be present. Melaniferous leucocytes may be found and during the leukopenia which follows the paroxysm the large mononuclears and transitionals may be increased to 20 per cent. However during the attack the leucocytes may definitely increase while in recovery the blood may contain numerous reticulocytes. There is a reduction in the alkalinity and coagulability of the blood. Haemoglobinaemia is common but not always found and may disappear early in the attack. Normal plasma not serum shows a haemoglobinaemia equal to 1 part red cells in 400 parts water. Pseudo methaemoglobin is especially prevalent in severe cases. In fatal cases it often rises progressively until death. Hyperbilirubinaemia is common with an indirect van den Bergh reaction varying from 5 to 88 units. Normal serum contains 0.2–0.5 units of bilirubin.

### DIAGNOSIS

**Clinical Diagnosis**—An unusually prostrating paroxysm similar to that of a malarial chill but with more intense rigor during which haemoglobinuria, early jaundice and marked bilious vomiting are features suggest the diagnosis of blackwater fever. The two diseases which are most likely to be confused with blackwater fever are yellow fever and bilious remittent malarial fever.

In Weil's disease the jaundice does not appear for 48 to 72 hours. The pulse is slow, there is no haemoglobinuria although there may be a haematuria and there is a polynuclear leucocytosis which may not occur in malaria. The van den Bergh test is often of value in differentiation. A case of paroxysmal haemoglobinuria occurring in a blackwater district may be impossible to differentiate from a very mild case of Blackwater fever. The means available for differentiation have already been referred to p. 148. Obviously any substance causing rapid haemolysis of blood may produce haemoglobinuria as chlorate of potash, carbolic acid poisoning, arseniuretted hydrogen, snake bite or severe burns. Fitz has seen it develop in patients receiving phenylhydrazine for the treatment of polycythemia.

### DIAGNOSIS

**Laboratory Diagnosis**—In the laboratory one may note evidences of malarial infection, rapid reduction in red cell count and haemoglobin

percentage According to the studies of Foy (1938) examination of the blood obtained by splenic puncture or from bone marrow puncture has been found to be no more valuable than the examination of the peripheral blood for the discovery of parasites

	Blackwater fever	Yellow fever	Bilious remittent
Onset	Sudden but asthenic with marked rigor	Sudden but asthenic for two or three days	Comes on more slowly
Urine	Haemoglobinuria Pink foam to urine Albuminuria from first day	No blood in urine before 3d or 4th day and then haematuria Albumin from 4th day	Bile in urine Yellow froth on shaking urine Albuminuria slight and not common
Icterus	Early and intense Comes on in a few hours	Does not appear before 3d day and gradually intensifies	Jaundice develops slowly about 2d day
Spleen	Somewhat enlarged and tender	No enlargement of spleen	Splenic enlargement is marked may have ague cake
Pulse	Rapid from start and becoming more so as disease progresses	Stationary pulse with rising temperature or falling pulse with stationary temperature (Faget's sign)	Pulse not so rapid as in blackwater
Vomit	Early marked bilious vomiting	Mucus like followed by black vomit about 4th day	Bilious vomiting and gastric distress less than in blackwater
Evidences of malaria	Frequently present as parasites or malarious leucocytes or increased large mononuclear percentage	Negative unless yellow fever occurs in a malarial case	Some evidence at some time almost always obtainable

It is difficult to make good blood smears of the thin blood the coagulation time of which is usually delayed The alkalinity of the blood is generally reduced The indirect van den Bergh and the spectroscopic changes of the pigments present (already discussed) also may be investigated The autolytic reaction is of assistance in excluding other forms of haemoglobinuria In this test take 5 cc of patient's blood in a small test tube place on ice for 5 minutes then incubate at 37 C for 1 hour No change occurs in blackwater fever while in paroxysmal haemoglobinuria intense haemolysis results

In the urine there may be noted the granular sediment of debris of red cell destruction with haemotoidin crystals at times. Red blood corpuscles may be entirely absent. Spectroscopically on treating the brown urine with caustic soda there may be observed the absorption bands of reduced haematin (haemochromogen). The characteristic bands of oxyhaemoglobin in severe cases and of methaemoglobin in mild cases may also sometimes be observed. Ammoniacal fermentation through contamination of the specimen may lead to the disappearance of methaemoglobin through the formation of alkaline methaemoglobin which does not present an alpha band in the red portion of the spectrum. However on acidification with acetic acid methaemoglobin reappears. Albumin is usually present in large quantity. Urobilin is usually present in the later stages in large amount. One can examine the urine for blood by the haemun crystals guaiac or benzidin tests. Burkitt has noted that his cases of blackwater have shown a very acid urine with large amounts of acetone bodies.

### PROGNOSIS

The prognosis is generally grave in severe cases. It is especially dependent upon the amount of red corpuscles destroyed and whether the kidneys continue to function. In cases of severe haemoglobinuria in which the urine is of a deep porter color the mortality is higher than when the haemoglobinuria is only mild.

Manson Bahr states that in southern Nigeria and in Algeria and in cases which have returned to London the case mortality has been as high as 50 per cent but as a general rule it may be estimated as about 25 per cent. Recent statistics available during the year 1938 show that in Japan the mortality reported by Akashi was 33 per cent by Krishnan in Calcutta 20 per cent (all deaths in Bengalese) while Blackie in southern Rhodesia reports in a series studied by Thompson a death rate of 23 per cent in 1054 cases and in another series by Ross of 20 per cent among 679 cases. Diederick who formerly collected statistics of various authorities found in cases which were treated by quinine a death rate of 25.9 per cent and in cases not so treated of 12.1 per cent.

Marked and persistent vomiting and hiccough are very unfavorable signs. In particular however it is anuria that gives us our greatest concern in the care of a case. Fairley says that an intense and progressively increasing jaundice associated with a rapidly rising bilirubin curve is undoubtedly ominous. However he found that death might also occur with relatively low values of bilirubin and only a very moderate degree of jaundice. In 7 fatal cases an indirect van den Bergh reaction was obtained varying from 5.0 to 88.5 units. A severe attack is followed by marked anaemia and convalescence is usually protracted.

A rare sequel is cholelithiasis due to the formation of biliary calculi from inspissation of bile in the gall bladder a case of which has been reported recently by Fairley and two by Manson Bahr. Manson Bahr also points out that haemorrhage into the retina sometimes occurs and

he has seen a case of altitudinal hemianopia where there was total blindness in the lower half of the visual field

One attack of blackwater fever appears to predispose to a second attack, and in Nigeria second attacks or more have occurred in about 20 per cent of the cases. When an individual recovers from 2 attacks the third is generally fatal.

After the patient has passed through an attack of blackwater fever active regeneration of the erythrocytes takes place. As a result there is sometimes marked polychromasia and polychromatophilic stippling of the cells. A larger number of normoblasts and of reticulocytes may appear in the peripheral blood. This has been regarded as a very good prognostic sign since it indicates that the haemopoietic organs are active. An increase of the mononuclear cells may also occur.

### PROPHYLAXIS AND TREATMENT

**Prophylaxis**—The view now generally entertained is that where malarial prophylaxis is properly carried out there should be little if any blackwater fever. In persons who have had a previous attack of blackwater fever and with whom quinine prophylaxis is decided upon quinine tannate or quinine base may be used, as it has been suggested that these preparations have a less destructive action on the blood corpuscles than the acid salts of quinine. Some prefer treatment with atebrian.

In particular any exposure to chilling influences or conditions which lower resistance should be avoided. Blackwater fever is more prevalent among those who have resided for 2 or 3 years in highly malarious tropical regions than among recent arrivals hence the former should especially exercise great care as to errors in diet, alcoholic excesses, exposure to wet and irregularity in quinine prophylaxis.

**Treatment**—There is little unanimity of opinion as to the advisability of giving quinine during an attack of blackwater fever. At present the possible danger of precipitating blackwater fever by the administration of quinine constitutes one of the chief responsibilities in the treatment of cases of malaria and especially of Caucasians in severely endemic malarious districts. It is on account of this danger that one cannot give definite advice for its treatment. It seems clear that in many individuals large doses of quinine may exercise a certain amount of destructive action upon the red blood corpuscles and disturb the physio-chemico-haemoglobin-red blood corpuscle balance. When the toxic influence of the drug is added to that of the parasites producing the disease it may be that their combined effect will result in a sudden extensive liberation of haemoglobin which might not have taken place had the quinine been withheld. It has been said that quinine base may exert a less haemolytic influence than the acid salts of quinine and it has also been suggested that atebrian is even superior for use in this connection. However, a number of malarialogists believe that the action of atebrian in influencing the onset of blackwater fever is probably the same as quinine.

It has been argued that any red cells containing parasites will almost surely be destroyed in the general haemolysis and with them the parasites they contain so that it may not seem reasonable to give quinine during the first day or two of the attack.

Bastianelli gave the following rules as to the use of quinine in haemoglobinuric fever (a) If haemoglobinuria occurs during a malarial paroxysm and parasites are found in the blood quinine should be given (b) If parasites are not found in the blood quinine should not be given (c) If quinine has been already given before the haemoglobinuria has appeared and no parasites are found its use should be suspended but if parasites persist it should be continued

DeLangen believes that while we may now and then have a haemolysis set up by the quinine this never presents a dangerous picture clinically and is certainly never fatal He believes that in general the fear of using quinine in a case of blackwater fever is grossly exaggerated and sometimes proves dangerous to the patient If quinine is given by mouth it should be administered cautiously so as not to produce or increase nausea or vomiting Manson Bahr prefers treatment with full doses of atabrin However he suggests that if this drug is not available small doses of quinine 1 gr twice daily should be given Such small doses are of doubtful value in the treatment of the malarial infection in adults

Absolute rest in bed avoidance of chilling and good nursing are prime considerations in treatment The patients should be given alkaline waters freely as Vichy or water containing 30 grains of bicarbonate of soda to the pint Adequate water excretion and the production (if necessary) of an alkaline urine is of importance in preventing the blockage of the tubules with haemoglobin infarcts and debris (see below) Cracked ice often tends to lessen the nausea and vomiting Albumin water or barley water may be retained better than milk or broths As the condition is so asthenic one cannot disregard the nourishment of the patient during the first 2 or 3 days as is true of the asthenic first stage of yellow fever Return of haemoglobinuria is often noted when regular diet is allowed for which reason fruit juices broths or milk should be continued well into the convalescent period

Hot fomentations to the loins are indicated for relief of pain and the effect on the renal congestion Saline enemata are of particular value and may suffice in mild cases In more serious ones proctoclysis by the Murphy drip method of giving fluids by rectum has been highly recommended for its effect upon the circulation and kidneys In severe cases subcutaneous or intravenous saline injections may be advisable Sorel recommends the intravenous injection of lactose or glucose solutions in quantities of about 300 cc (Crystallized glucose 47 grams water 1000 cc or C P lactose 92.5 grams water 1000 cc) He also uses these sugar solutions as enemata

Manson Bahr has also found valuable intravenous injections of 5 per cent glucose in warm saline a pint at a time If there is threatened



suppression of the urine dry cupping or heat fomentations over the loins may be employed and caffeine citrate 2 gr a day may be given as a bland diuretic. As there is certain clinical and experimental evidence to indicate that suppression of urine is much less likely to occur if the urine is alkaline sodium citrate together with sodium bicarbonate may be given until an alkaline reaction of the urine is obtained. It is sometimes necessary to give as much as 1 drachm of sodium bicarbonate at 4 hourly intervals. In very severe cases intravenous injections of sodium bicarbonate 150 grains to a pint of distilled water, may be administered. Hanschell has advised that not more than one pint at a time (500 cc) should be injected on account of the danger of bringing about an oedematous condition of the lungs. High rectal enemata of hot water will sometimes give rise to diuresis. Burkitt has reported excellent results by the intravenous injections of alkaline salts while Hearsey also advocates a mixture in which there is 10 grains bicarbonate of soda to  $\frac{1}{4}$  grain bichloride of mercury in each dose, to be given every 2 hours. For the urinary suppression Wallace particularly recommends salines as hot as can be born, administered high in the colon by a double flow tube. Since he found this treatment effective after intravenous and rectal injections had failed, it is inferred that the results obtained were due particularly to the action of the heat applied to the splanchnic area rather than to the further administration of fluid.

Turpentine stupes and mustard plasters to the epigastrium may aid in control of vomiting. Restlessness may require minute doses of morphia. Calomel in large doses has been recommended by some clinicians but it would seem more advisable only to use calomel to keep the bowels open and then in small divided doses.

Antipyretics should not be used, because of their depressing action on the heart. However, cardiac stimulants are frequently indicated and caffeine is especially valuable both as a diuretic and heart stimulant.

Among special drugs that have been used, cholesterol has been given in 15 grain doses in suspension in thick milk every 4 hours with the idea that it might be anti haemolytic. Krishnan (1937) has found colloidal solution of cholesterol of some value in the experimental treatment of malarial infection in monkeys. In the treatment of monkeys he has also obtained encouraging results with a combination of glucose ascorbic acid and cortin. The use of cortin appeared especially important and was given to counteract degenerative changes observed in the adrenal cortex in fatal cases.

Transfusion of blood has been practised with good results though reports of such treatment in some cases has indicated that while temporary improvement may occur yet this may be followed by a return of haemoglobinuria. Blackie (1937) has employed blood transfusions for 5 years upon 50 cases of blackwater fever in 22 of which the treatment was controlled throughout the whole of the illness. Fifteen of the 22 cases were treated in hospitals and the remaining in the patients homes. Thirteen of the 15 hospital patients recovered and 6 of the 7 country cases. Three

deaths occurred in the patients suffering from anuria. He believes that blood transfusion is an important life saving measure in this disease and that if its full value is to be derived it must be instituted in the early stages of the disease. But while blood transfusion is not indicated in every case of blackwater fever it is especially useful in the toxic polyuric and in the relapsing type of case and in post blackwater asthenia. However it is definitely contraindicated in toxic anuric blackwater fever. Manson Bahr believes that in severe cases transfusion of compatible blood should be employed from the moment haemolysis commences. Considerable care must be exercised in grouping the blood as in a haemolytic condition the corpuscles are prone to auto agglutination. Boyle (1942) also emphasizes the value of transfusion in the early stages of cases in West Africa. Dameshek (1940) has pointed out the quick recovery that often follows a single transfusion of blood in haemolytic anaemias which indicates that normal serum contains an anti haemolytic factor.

As an aid to recovery during convalescence neosalvarsan has been recommended. Both DeLangen and Fairley point out that iron preparations are not indicated in this disease on account of the great amount of iron already available in the body. Arsenic appears to be preferably indicated for the treatment of the anaemia which results.

**Action of Synthetic Drugs**—The possible action of synthetic drugs in producing attacks of blackwater fever is of great importance. The large amount of clinical data already available has failed to clear up this problem. A few years ago the successful use of plasmoquine in the treatment of blackwater fever was reported by Muhlens and Fischer, Memmi and Schulemann, Cooke and Willoughby and others. Brosius however found that plasmoquine does not prevent the development of blackwater fever.

Amy and Boyd (1936) in India appeared to attribute to the use of plasmoquine a notable increase of cases of haemoglobinuria in certain regions. Owing to its methaemoglobin producing action plasmoquine should probably be regarded as one of the specific drugs capable of bringing on attacks of haemoglobinuria. However Fairley and Bromfield emphasize that haemoglobinuria in blackwater fever is not an outcome of a single pathogenic process and that various pigments are produced in the disease. It appears certain that methaemoglobinuria consecutive upon the use of plasmoquine is in part of intracorpuscular origin whereas Fairley believes that in blackwater fever it is of extra corpuscular origin and that the haemoglobinuria which occurs is generally preceded by a change in the oxyhaemoglobin after liberation into the blood plasma of the latter pigment through haemolysis.

A few cases of blackwater fever following the use of atebrian in conjunction with plasmoquine have also been observed. Bannerjee at Brahmachari (1933) reported a case in which the attack of blackwater fever occurred after 5 days treatment with 0.20 grm atebrian and 0.02 gm plasmoquine per day. Chopra and Chaudhuri recounted a similar case after such treatment taken by the patient himself. On the other hand Das Gupta has reported a case of blackwater fever which was successfully

treated with atabrin in which quinine had not been successful and Manson Bahr (1938) considers atabrin the drug of choice

The use of atabrin in the treatment of blackwater fever has also been emphasized by McNabb and Schwartz (1934) in the Philippines Goldblatt (1935) in South Africa, and Maldonado (1936) in Spain, and they have reported satisfactory results. However these observations do not afford evidence of the harmlessness of the drug in question as a possible factor in attacks of haemoglobinuria. Many cases of blackwater fever have also been treated with quinine, in which the patient's life has been saved although it is generally recognized that this drug may conduce to the onset of blackwater fever in certain individuals. Christophers (1937) has emphasized that the precipitation of blackwater fever by quinine seems to be independent of the normal toxic effects of this drug and except with certain definitely quinine haemoglobinuria susceptible individuals occurs in such an unpredictable manner that a similar effect with atabrin could only be excluded as a result of a great deal of experience. So far cases of blackwater fever reported after the administration of atabrin have been few. Foy observed 4 in Greece while Ciuca reported that in a patient inoculated with *P. knowlesi*, in whom a severe infection resulted a single dose of 0.30 grm. of atabrin was followed by an attack of methaemoglobinuria which lasted 5 days. In this instance also it is necessary of course to consider the distinction between the real blackwater fever methaemoglobinuria and that perhaps brought about by the drug due to its methaemoglobinizing effects as may occur with plasmoquine.

Manson Bahr (1940) notes that blackwater fever may ensue after a course of atabrin, as has been noted by several observers. He has seen one striking case of this, but believes the general impression prevails that it is of less frequent occurrence than after quinine.

There seems to be general agreement that especially when used unsystematically all antimalaria drugs are apt to disturb the physio-chemico Hb. red corpuscle balance in the organism which is already invaded by parasites and may already have been rendered susceptible by other pathological factors. Hence having in view the methaemoglobin producing action of plasmoquine and the few reports of cases of haemoglobinuria following the use of this drug together with atabrin plasmoquine should certainly be avoided in individual treatment of any cases in which the condition or history of the patient would contraindicate its use. The question of to what extent atabrin can be safely used in the treatment of blackwater fever is still uncertain. Some authorities believe that the action of atabrin in influencing the onset of blackwater fever is probably the same as quinine.

A few reports have been made that liver extract appears to be of great benefit if given with atabrin (Chandler, 1940).

In connection with treatment it should be emphasized that it is especially dangerous for a person who has had one attack of blackwater fever to return to a country where malaria prevails. It has also been

found dangerous to transport suddenly from the tropics to a cold climate a patient who has had an attack of blackwater fever

Circular Letter No 36 War Dept. Office of the Surgeon General Washington 1942 recommends the following treatment

- (1) Do NOT give quinine until convalescence from the attack of blackwater fever is established.
  - (2) Absolute rest in bed. Keep patient warm.
  - (3) Give a minimum of 2000 cc. of fluids per day much more if possible.
  - (4) During the period of vomiting if urine is acid or azoturia exists give 1000 cc. of normal saline or of 5 per cent glucose. This can be repeated after 12 hours if urine remains acid.
  - (5) When vomiting is controlled give sodium bicarbonate 0.6 gram (10 grains) by mouth every 1 to 2 hours until urine is alkaline to litmus thereafter give only if urine becomes acid.
  - (6) If unable to void catheterize every 4 hours in order to determine urine output and reaction to litmus.
  - (7) For severe anemia give transfusions repeated daily as needed.
  - (8) After convalescence is established if plasmodia are present in the blood give atabrine 0.1 gram three times daily for 5 days. Watch for occurrence of haemoglobinuria as atabrine has occasionally precipitated an attack.
- Prognosis—(1) Treat every case of estivo autumnal malaria to complete cure.  
 (2) Recurrence of blackwater fever is common especially in the tropics. Send patient to temperate zone if possible.

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treated with atebirin in which quinine had not been successful and Manson Bahr (1938) considers atebirin the drug of choice

The use of atebirin in the treatment of blackwater fever has also been emphasized by McNabb and Schwartz (1934) in the Philippines Goldblatt (1935) in South Africa and Maldonado (1936) in Spain and they have reported satisfactory results. However these observations do not afford evidence of the harmlessness of the drug in question as a possible factor in attacks of haemoglobinuria. Many cases of blackwater fever have also been treated with quinine in which the patient's life has been saved although it is generally recognized that this drug may conduce to the onset of blackwater fever in certain individuals. Christophers (1937) has emphasized that the precipitation of blackwater fever by quinine seems to be independent of the normal toxic effects of this drug and except with certain definitely quinine haemoglobinuria susceptible individuals occurs in such an unpredictable manner that a similar effect with atebirin could only be excluded as a result of a great deal of experience. So far cases of blackwater fever reported after the administration of atebirin have been few. Foy observed 4 in Greece, while Ciuca reported that in a patient inoculated with *P. knowlesi* in whom a severe infection resulted a single dose of 0.30 grm. of atebirin was followed by an attack of methaemoglobinuria which lasted 5 days. In this instance also it is necessary of course to consider the distinction between the real blackwater fever methaemoglobinuria and that perhaps brought about by the drug due to its methaemoglobinizing effects as may occur with plasmoquine.

Manson Bahr (1940) notes that blackwater fever may ensue after a course of atebirin as has been noted by several observers. He has seen one striking case of this but believes the general impression prevails that it is of less frequent occurrence than after quinine.

There seems to be general agreement that especially when used unsystematically all antimalaria drugs are apt to disturb the physico-chemico Hb. red corpuscle balance in the organism which is already invaded by parasites and may already have been rendered susceptible by other pathological factors. Hence having in view the methaemoglobin producing action of plasmoquine and the few reports of cases of haemoglobinuria following the use of this drug together with atebirin plasmoquine should certainly be avoided in individual treatment of any cases in which the condition or history of the patient would contraindicate its use. The question of to what extent atebirin can be safely used in the treatment of blackwater fever is still uncertain. Some authorities believe that the action of atebirin in influencing the onset of blackwater fever is probably the same as quinine.

A few reports have been made that liver extract appears to be of great benefit if given with atebirin (Chandler 1940).

In connection with treatment it should be emphasized that it is especially dangerous for a person who has had one attack of blackwater fever to return to a country where malaria prevails. It has also been

found dangerous to transport suddenly from the tropics to a cold climate a patient who has had an attack of blackwater fever

Circular Letter No. 56 War Dept. Office of the Surgeon General Washington 1942 recommends the following treatment

- (1) *Do NOT give quinine* until convalescence from the attack of blackwater fever is established
  - (2) Absolute rest in bed. Keep patient warm
  - (3) Give a minimum of 2000 cc. of fluids per day much more if possible
  - (4) During the period of vomiting if urine is acid or anuria exists give 2000 cc. of normal saline or of 5 per cent glucose. This can be repeated after 12 hours if urine remains acid
  - (5) When vomiting is controlled give sodium bicarbonate 0.6 grams (10 grains) by mouth every 2 to 3 hours until urine is alkaline to litmus thereafter give only if urine becomes acid
  - (6) If unable to void catheterize every 4 hours in order to determine urine output and reaction to litmus
  - (7) For severe anemia give transfusions repeated daily as needed
  - (8) After convalescence is established if plasmodia are present in the blood give atabrine 0.1 gram three times daily for 5 days. Watch for recurrence of hemoglobinuria as atabrine has occasionally precipitated an attack
- Prognosis*—(1) Treat every case of estivo-autumnal malaria to complete cure  
 (2) Recurrence of blackwater fever is common especially in the tropics. Send patient to temperate zone if possible

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## Chapter III

# AFRICAN TRYPANOSOMIASIS

### SYNONYMS AND DEFINITION

Synonyms —Sleeping sickness, Negro lethargy French *Maladie du sommeil* German *Schlafkrankheit*

Definition —African trypanosomiasis is a specific febrile infection often characterized by weakness wasting and a protracted lethargy or a soporose condition. It is a protozoan disease caused by a flagellate *T. gambiense* (var *T. rhodesiense*) and transmitted by species of tsetse flies of the genus *Glossina* (especially *G. palpalis*, *G. tachinoides*, *G. morsitans* and *G. swynnertonii*). The trypanosomes are blood parasites not only in man but also in some wild and domestic animals which may serve as reservoirs of the disease. A period of fever is followed by an inflammatory condition of the lymphatic system often leading to adenitis areas of oedema and a meningo-encephalitis. Symptoms of the latter are dullness of the intellect apathy physical and mental lethargy associated with tremors and peculiar gait and sometime mania. Unless treated the disease usually ends fatally.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

The scourge of trypanosomiasis which affects both man and domestic animal, has been one of the black clouds which has overhung the continent of tropical Africa. Not only the ravages of the human disease itself but the fear of contracting it and the enormous economic losses which have resulted from destruction of live stock have for years interfered with successful colonial development of vast tracts of the continent.

Sleeping sickness the terminal stage of human trypanosomiasis was known for centuries before the discovery of its causative factor the trypanosome. The slave traders in earlier years came to recognize the symptoms of lethargy among certain Negroes and the risks and high mortality of the affection. They also discovered that the swollen glands of the Negro were a symptom of the affection and hence refused to buy Negroes with enlarged glands. Livingstone, in 1849 was familiar with the tsetse fly and the fly disease of cattle but apparently he did not recognize the human disease and Mense has believed that sleeping sickness was not then present in East Africa but was introduced by natives of Stanley's expedition. It seems evident that Stanley's expedition to reach Emin Pasha in 1888 probably introduced sleeping sickness into virgin territory in Uganda and the region of the great lakes where it gave rise to the terrible epidemic that destroyed in one district two

thirds of the population in 8 years some whole villages and islands being depopulated. In recent years the severity of the disease has been greatly reduced in many parts of Africa by protective measures and by examination and treatment on a very large scale. In Nigeria alone from 1931 to 1937 over 2 000 000 examinations were made and 300 000 new cases diagnosed.

Scott (1939) and Kirk (1940) have given evidence of the description of sleeping sickness by A. L. Qualquasandi an Arab writer of the 14th century.

The case referred to was that of Mari Jaza a sultan of the Mali kingdom whose condition was described as follows — His end was to be overtaken by the sleeping sickness (il t an nawm) which is a disease that frequently befalls the inhabitants of those countries especially their chieftains. Sleep overtakes one of them in such a manner that it is hardly possible to awake him.

John Atkins a British naval surgeon in 1734 on his return from West Africa described sleeping sickness as it occurred on the Guinea coast —

The Sleepy Distemper (common among the Negroes) gives no other previous Notice than a want of Appetite 2 or 3 days before their sleeps are sound and Sense and Feeling every little for pulling drubbing or whipping will scarce stir up Sense and Power enough to move and the Moment you cease beating the smart is forgot and down they fall again into a state of Insensibility driving constantly from the Mouth as if in deep salvation breathe slowly but not unequally nor snort. Young people are more subject to it than the old and the Judgment generally pronounced is Death the Prognostick seldom failing. If now and then one of them recovers he certainly loses the little Reason he had and turns Idiot.

Winterbottom in his African travels in 1803 mentioned the disease under the name of *kondee* and called attention to the enlargement of the posterior cervical glands in the disease which has come to be known as Winterbottom's sign. In 1879 Lewis in Calcutta first described the mammalian species of trypanosome in the blood of a rat *Trypanosoma lewisi* now known to be transmitted among rats by the rat flea *Ceratophyllus fasciatus* or the rat louse *Haematopinus spinulosus*. Evans (1880) found a similar parasite *Trypanosoma evansi* to be the cause of *surra* a disease of horses in India. In 1895 Bruce in South Africa showed that *nagana* a similar disease affecting both horses and cattle was also due to a species of trypanosome *T. brucei* and that as had been suspected the infection was transmitted from animal to animal by the bite of a tsetse fly *Glossina morsitans*.

Up to 1890 trypanosomes had been found only in animals and not in man. In 1890 Nepveu found a trypanosome in the blood of a man in Algeria but owing to the nature of the description his report did not receive recognition. In 1901 and 1902 Ford and Dutton discovered a trypanosome in the blood of a patient in Gambia with a peculiar irregular fever and named it *Trypanosoma gambiense*. In 1902 Castellani found a trypanosome in the cerebrospinal fluid in 5 cases of sleeping sickness in one of which the parasite was also present in the blood thereby establishing a connection between the febrile stage with a trypanosome in the

blood and the sleeping sickness stage with a trypanosome in the cerebrospinal fluid. He named the parasite *T. ugandense* but it was subsequently shown to be identical with *T. gambiense*. The following year, Bruce and Nabarro confirmed these observations and showed that the disease was spread by another tsetse fly, *Glossina palpalis*. At first they considered the transmission by fly as purely mechanical but Kleine afterwards showed that the parasite undergoes true development in the body of the tsetse fly, a fact later confirmed by Bruce, Robertson, Taute, Mackie and others. In 1910 Stephens and Fantham described as the cause of Rhodesian, or a more acute form of sleeping sickness a new species *Trypanosoma rhodesiense*. Kinghorn and Yorke in 1912 showed this organism to be transmitted by *Glossina morsitans*.

**Geographical Distribution**—African sleeping sickness was apparently first reported from Sierra Leone. It was also noticed early in the 19th century in Liberia the local name of 'konje kira' being applied to it there. Doala Bukere the inventor of the Vai alphabet died from the disease. It has been stated that it was imported from the West Coast of Africa to the West Indies on several occasions probably by the slave trade but soon died out obviously owing to the absence of the tsetse flies. At the present time it exists on the West Coast of Africa from Senegal to Mossamedes in Angola up to Timbuctu on the Niger throughout the Congo into Uganda Rhodesia South Nyasaland and Portuguese East Africa from Uganda and Busera southward to former German East Africa and Lake Tanganika and northwards to the Bahr El Ghazal province.

Trypanosomiasis reported due to *Trypanosoma gambiense* has a much wider distribution than that reported to be due to *T. rhodesiense*. The geographical distribution of the disease caused by *T. gambiense* corresponds roughly to the distribution of *Glossina palpalis* while the form of the disease ascribed to *T. rhodesiense* corresponds to part of that of *G. morsitans*. The latter is found especially in Northeastern Rhodesia particularly in the Luangwa Valley, about the southernmost limit, 14° S in the southeastern portions of Tanganyika Territory up to 10° S in Portuguese East Africa and in Nyasaland especially in the region south and west of Lake Nyasa and the southern Sudan.

The severity and prevalence of sleeping sickness differs greatly in the endemic areas and the disease shows a curiously localized incidence at times developing in one area yet failing to occur in closely adjacent territory where the tsetse fly is found hence there may be wide areas which are entirely or almost free of infection separating heavily stricken tracts of country. Thus in some communities 90 per cent of the population is infected. In others only 4 or 5 per cent will show infection. In some sections with the advent of exploration and advancing civilization the disease has done great damage in epidemic form. In other places where the disease has long existed, the mortality is much less. The capricious distribution along the water courses depends upon the breeding habits of the vectors the tsetse flies all species of which are

limited to Africa and African sleeping sickness occurs endemically only in that continent. A few cases of trypanosomiasis have been discovered in the New World particularly among laborers from the West Coast of Africa. Some of these have been reported upon by Guérin in Martinique. Numbers of cases have been observed and treated during recent years in the United States in individuals who have contracted the infection in Africa. Other cases of this nature have been occasionally detected and treated in various capitals and schools of tropical medicine in Europe.

**Prevalence**—Trypanosomiasis has dominated and seriously interfered with colonial development in about one fourth of the continent of Africa. Discovered in Uganda in 1900 it was estimated that it killed in the affected areas 200 000 of a population of 300 000. A recent report of the League of Nations states that over 1 000 000 natives are treated for sleeping sickness every year. In regard to the incidence and geographical distribution of the disease in British tropical Africa Granville Edge (1938) writes that there were 70 830 cases of human trypanosomiasis in 1936 recorded in British hospitals in West Africa. Roughly 5 per cent of the cases and 5 per cent of the deaths were due to sleeping sickness. In Gambia the most westerly of the West African possessions over 40 per cent of the inpatients and 30 per cent of the hospital deaths were ascribed to sleeping sickness during 1936. These facts are of special significance since in 1923 only 3 cases of the disease were recorded and death from trypanosomiasis was said to be seen only occasionally. However since then the disease has steadily increased and travellers have reported a marked increase in tsetse flies in such areas.

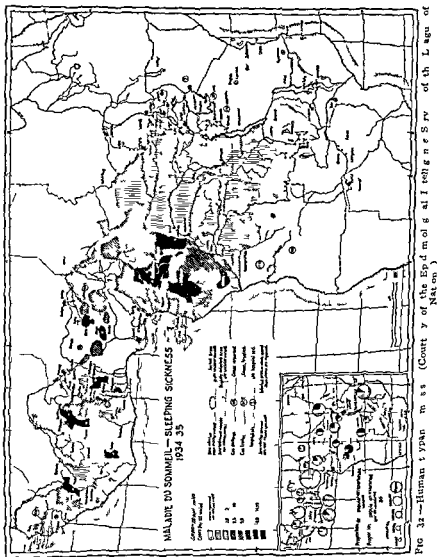
A hyper endemic area extends from MacCarthy Island along the Gambia River and in a hospital about 70 miles from Bathurst some 900 patients with sleeping sickness were treated during 8 months of the year.

In Sierra Leone and in Liberia the disease appears to be of no great importance and only a few sporadic cases have been seen in recent years. However on the Gold Coast sleeping sickness continues to be a cause of serious anxiety. During the past 5 years cases have increased from 685 to 4826 and the deaths from 45 to 182. During 1936 10 cases occurred among Europeans.

In the largest of the British West African colonies Nigeria (total population about 20 millions) MacQueen (1938) reports that the total number of diagnoses made from 1931 to 1936 was 264 933 and Lester (1939) reports that 300 000 cases were treated during the years 1932-39. Over 60 000 cases of sleeping sickness were treated during 1936 and 69 hospital deaths were described. Hence it would appear that in sleeping sickness this country has a public health problem of magnitude. In the northern provinces its spread has reached alarming proportions. During 1936 in these provinces 6 sleeping sickness teams of men examined 417 495 people and of these 47 550 or 11.4 per cent were found to be infected. The Report of the Nigerian Sleeping Sickness Service 1938 states that during the year 378 109 were examined and 35 006 found infected. In 1939 485 000 were examined and approximately 10 000 diagnosed and treated. In North Dahomey from 1932 to 1936 Beaudes observed 7701 new cases. MacQueen believes that in Nigeria sleeping sickness has become more virulent during the past 6 or 7 years in some localities where *G. morsitans* is not present and there is little game.

In the Belgian Congo the disease prevails in the Kwango and Kasai and Semliki districts. Schwetz reports that in the Kwango and Kasai districts 45 000 cases were detected in a population of 550 000. In the Kwango area in 1937 10 258 new cases

were treated. Kisanu has been a heavily infected region where two thirds of the population was said to have died of the infection within 10 years. In the French African territory G. Martin in the examination of half a million inhabitants found 28 500 cases or about 5 per cent of the population. Blanchard and Laigret found the disease prevailing in the upper Ogowe region in Gabon 30 per cent of the inhabitants



being infected. In the Chad colony the infections were estimated at 7 per cent of 1 500 000 inhabitants with an average yearly death rate of 25 000. In the Cameroons Tamon and Jamot found that of 100 000 natives examined through several years 30 000 were infected the percentage in the different districts varying from 8 to 45 per cent. In 1928 the writer found the fertile Semliki Valley between Lakes Albert and

Edward almost entirely evacuated on account of the previous ravages of sleeping sickness

On the eastern side of Africa in 1936 some 2770 cases and 452 deaths were ascribed to sleeping sickness in the British possessions. In the Sudan in 1933 some 855 cases were treated but owing to preventive measures in 1936 only 150 cases none of which were fatal were reported and in 1938 only 89 cases. The disease is found only in the southern part of the Equatorial Province and *G. palpalis* is not found north of this region.

In Uganda in 1900 a terrible epidemic of the disease was discovered in the Lake Victoria region. The inhabitants of the Boruma Islands numbered 56 000 in 1900 and had decreased to 13 000 in 1907. The total population of the infected districts in Uganda fell from 300 000 to 100 000. The epidemic was finally stopped by removing the remaining population from the shores of Lake Victoria Nyanza and the islands. Later however a second epidemic occurred in these regions and Duke reported that in 1918 the death rate from sleeping sickness in this region was 428 per thousand.

In 1936 in Uganda there were only some 2000 cases and 58 deaths recorded. The incidence of the disease had shifted from Lake Victoria to the regions of the River Koirich in the west Nile district. Very few cases were detected in the Lake Edward George Albert areas and it is believed that sleeping sickness will shortly be eradicated from them. On the other hand a large increase in the incidence of the disease is reported in the west Nile district with 1876 cases and 54 deaths. Farther east in Kenya the disease is sporadic so that only 11 cases with 2 deaths were recorded in 1936. South of Kenya in Tanganyika Territory with a population of over 5 millions 536 cases were diagnosed with 384 deaths during the year. In Northern Rhodesia in which the disease is endemic in the north only 30 cases and 5 deaths were reported. In Nyasaland where the tsetse fly is widely distributed only 14 non fatal cases were detected during the year.

Although the disease is usually common in the natives it is comparatively rare among white people in Africa.

In connection with the distribution of human trypanosomiasis as illustrated in the accompanying map the Epidemiological Service of the League of Nations points out that

In African territories comprised within the tropics (with an estimated population of 65 millions) nearly 7 million inhabitants were examined in the course of a single year (1934-35) and 140 000 new patients were seen and treated in addition to an approximately equal number of old patients.

In territories where the campaign has already been going on for some time (Belgian Congo French Equatorial Africa Cameroons) the proportion of the population examined each year is considerable amounting to 6 out of 15 million inhabitants (black areas in the diagram relating to examinations of the population).

Recent prospection in the colonies situated to the west of the Cameroons shows that sleeping sickness holds a much more important place in the pathology of West Africa than was hitherto suspected.

## ETIOLOGY AND EPIDEMIOLOGY

**Zoology**—The parasite of sleeping sickness is classified in the Order Flagellata (*Mastigophora*). In this class of protozoa the adults have flagella for the purposes of locomotion and the obtaining of food.

Some flagellates more or less resemble rhizopods in being amoeboid and in having an ectoplasm and an endoplasm. The body is frequently covered by a cuticle (periplast). Some flagellates have a definite mouth part the cytostome which leads to a blind oesophagus, others absorb food directly through the body wall. In addition to flagella some flagellates possess an undulating membrane. All flagellates possess a nucleus and some have contractile vacuoles. The flagellum may arise directly from the nucleus or from a small kinetic nucleus the blepharoplast (micronucleus or basal granule).

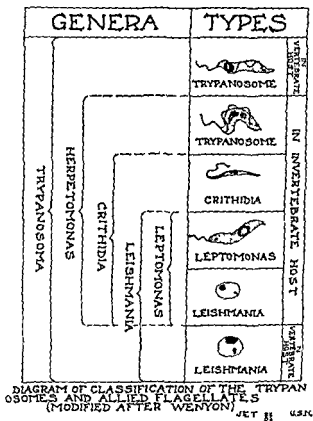


FIG 33 - Trypanosomida

The 2 important flagellates which are of serious pathogenic importance to man are the haemoflagellates of the genera, (1) *Trypanosoma* and (2) *Leishmania*. In addition flagellates occur in the intestine and in the vaginal secretion of the genera *Giardia* and *Trichomonas*.

*The Trypanosomidae*—The members of this family are probably primarily insect parasites some of which have become partially adapted to vertebrates or to plants.

There are 4 morphological types (1) leishmania (2) leptomonas (3) crithidia and (4) trypanosome (see Fig 33). The most primitive form is the leptomonas from which the others are derived. In this type the body is elongated and the kinetoplast from which the flagellum arises is near the anterior end and hence there is no undulating membrane. In the crithidia the kinetoplast is near but still anterior to the nucleus. The axoneme of the flagellum passes from the kinetoplast to the convex margin of the body and thence along its surface or on the edge of an undulating membrane to the anterior end of the body and becomes the flagellum. The free edge of the membrane is longer than the attached margin hence the membrane is thrown into folds. In the trypanosome form the kinetoplast is near the posterior end and the axoneme passes along an undulating membrane from the kinetoplast to the anterior end of the body. A free flagellum may or may not be present. It is also customary to refer to a metacyclic form. The metacyclic trypanosome occurs at the end of the cycle in the insect and resembles the form found in the blood of mammals but is usually smaller. In the case of *T. gambiense* the fly does not become infective for mammals until the salivary glands have become invaded by the metacyclic trypanosome. In the leishmanial form the nucleus and the kinetoplast are contained in a small round body and the axoneme extends from the kinetoplast to the periphery of the body. This form may be assumed by any of the preceding types.

On the basis of their morphology and life cycle Wenyon has differentiated 6 genera (1) *Leptomonas* (2) *Crithidia* (3) *Herpetomonas* (4) *Phytomonas* (5) *Leishmania* and (6) *Trypanosoma*. The members of the first 3 genera are intestinal parasites of invertebrates and are transmitted by encysted forms in the faeces. The members of the fourth genus *Phytomonas* are similar to the *Leptomonas* morphologically but require both plants and invertebrates in their life cycle. The members of the last 2 genera *Leishmania* and *Trypanosoma* (the haemoflagellates) are communicated to man and other vertebrates by the bites of certain arthropods. The *Leishmania* occur only in the leishmanial and leptomonas forms like the *Leptomonas* from which they are differentiated by the fact that they have a vertebrate as well as an invertebrate host in which either phase may occur. The *Trypanosoma* resemble the *Herpetomonas* in that all 4 forms exist but differ in that they have both vertebrate and invertebrate hosts.

*Genus Trypanosoma* Gruby (1843).—This genus was established by Gruby for flagellates found in frogs. The members of the genus are parasites of the blood or tissues of vertebrates. At some stage in their life cycle they have the typical trypanosome structure the body being elongated and containing a nucleus kinetoplast undulating membrane and a single free flagellum. An insect as well as a vertebrate host is known for many of the species and transmission is either through the mouth parts of the intermediate host in the act of sucking the blood of the definitive host or by the vertebrate ingesting infected faeces of the intermediate host. An exception is *Trypanosoma equiperdum* causing the disease known as dourine in horses where transmission so far has only been reported through coitus. Development of the trypanosome in the invertebrate host may lead to infection of its mouth parts transmission then occurring by the bite of the arthropod in which case the trypanosomes are said to have an anterior station. If development of the parasite is limited to the intestine and the trypanosomes are found only in the faeces transmission may occur by the ingestion of the latter or by scratching the parasites into the wound made by the bites of the insect. In this case the trypanosomes are said to have a posterior station.

The flagellates of the Genus *Trypanosoma* which infect man are *T. gambiense* (var *rhodesiense*) and *T. cruzi*. Those causing African sleeping sickness *T. gambiense* and *T. rhodesiense* are transmitted by the bites of *Glossina* flies and are anterior station trypanosomes while *T. cruzi* causing South American trypanosomiasis which is transmitted by rubbing the infected faeces of the bug *Triatoma* into the wound made by the bite



has a posterior station. The flagellates of the Genus *Trypanosoma* which cause sleeping sickness in man appear in the blood in trypanosome form and undergo in insects a cycle of development in which crithidia and metacyclic trypanosomes arise.

Recently there has been an attempt made by Jacono (1938) and Swartzweller (1938) to classify the mammalian trypanosomes using the position of the kinetoplast for their separation into 2 genera (1) *Trypanosoma* in which the kinetoplast is situated near the nucleus and (2) *Castellanella* in which it lies near the posterior end of the body (terminal or subterminal). Hoare (1938) in a careful study of the differentiation of the kinetoplast in the mammalian trypanosomes has shown that Jacono's classification cannot be applied to mammalian trypanosomes since none of the 5 groups can be distinguished by the position of the kinetoplast.

# DIFFERENTIAL CHARACTERS OF THE KINETOPLAST IN MAMMALIAN TRYPANOSOMES

Group	Species	Characteristics of kinetoplast							
		Size		Shape			Position		
		Diameter (μ)	Area (μ <sup>2</sup> )	Rounded		Rod (°)	Sub-terminal (°)	Sub-terminal (°)	Terminal (°)
				Crithidia (°)	Oblong (°)				
Lew	T. l. wis.		0.8	5	85	—	—	100	—
	T. th. l. r.	1.1	0.9	76	24	—	—	10	84
	T. cru.	2	1	70-8	10-30	—	—	0	—
	T. vot. mys.	1	8	84	16	—	—	100	—
	T. m. loph. g. m.	1.4	1.5	—	+	+	+	—	+
V. ax.	T. viva.	1	9	30-52	48-70	—	—	24-60	31-76
	T. l. m.	1	0.9	35-54	46-62	—	—	26-35	65-74
Congl. s.	T. c. g. l.	0.7	0.4	20-45	10-40	15-70	—	86-93	7-14
	T. s. m.	0.8	5	52	26	22	—	99	1
Bru.	T. bru.	0.6	0.3	3	—	75	—	74	4
	T. bod. na.	0.6	0.3	11	—	80	—	74	3
	T. g. mbi. se.	0.6	0.3	5	—	95	—	62	8
Ev. s.	T. evan.	0.2	0.4	17-40	7-2	48-76	—	91-99	9
	T. qu. pe. d. m.	0.6	3	5	—	75	—	00	—

*O. lytw. sp. c. m. s. f. th. sp. c. t. ere. l. bl.*  
*T. blasfom. C. A. Ho. D. S. (fom. W. H. m. B. a. of S. l. f. c. Re. rch. L. d.)*

Blacklock has suggested that a convenient means of classification of the flagellates of the genus *Trypanosoma* is offered by the character of the flagellum. (1) If all the individual trypanosomes of the species possess a free flagellum the species is monomorphic e.g. *T. cruzi*. (2) If none of the individuals possess a free flagellum the species is again monomorphic. No example is known in man. (3) If some individuals

of the species possess while others do not possess a free flagellum the species is polymorphic e.g. *T. gambense*, *T. rhodesiense* and *T. brucei*. In this classification obviously it is not variation in shape or in the size of the body which indicates a trypanosome is monomorphic but simply the flagellar character. Only individual parasites which are not undergoing division should be classified by this character.

### IMPORTANT TRYPANOSOMES OF ANIMALS

*Trypanosoma brucei*—This trypanosome is polymorphic and causes a fatal disease in horses and one from which few cattle recover. It is called nagana or the fly disease because it is transmitted by the tsetse fly *Glossina morsitans*. All mammals are probably more or less susceptible. The disease is characterized by fever, oedematous areas about the neck, abdomen and extremities, progressive anaemia and emaciation. It is an important disease of domesticated animals in many parts of Africa.

*Trypanosoma evansi* is a monomorphic trypanosome possessing a flagellum. It is the cause of a very fatal disease of horses in India and the Orient known as surra. It also affects camels and sometimes cattle. It is believed to be transmitted mechanically by biting flies (*Stomoxys*). The symptoms are fever, emaciation, oedematous areas and great muscular weakness. It was present in mules in the Philippine Islands in 1941.

*Trypanosoma equinum*—This trypanosome causes a fatal disease in horses in South America. There is paralysis of the hind quarters of the horse which gives the disease the name mal de caderas. It resembles *T. evansi* morphologically except that only the blepharoplast of the kinetoplast is present, the parabasal body not being distinguishable. Such forms in other trypanosomes may be produced experimentally by the action of drugs. *T. equinum* may be transmitted mechanically by *Stomoxys calcitrans*. It has been reported also in the capibara (*Hydrochoreus capibara*).

*Trypanosoma equiperdum*—This trypanosome also of the *T. evansi* type causes a disease of horses in many parts of the world. It is known as duourine and is transmitted especially by coitus. The genital organs show marked oedema which is followed by anaemia and paralysis. Mechanical transmission by *Stomoxys calcitrans* and *Tobanus nemoralis* has also been shown.

*Trypanosoma congolense* (*T. dimorphum*)—This trypanosome causes a disease in horses, cattle, sheep, goats, pigs and dogs in many parts of Africa while game may serve as reservoirs. It is transmitted by several species of *Glossina*. *T. congolense* is the smallest of the pathogenic African trypanosomes. It varies in length from 9–12  $\mu$ . Its breadth is under 3  $\mu$ . Present in horses in Arizona, Southern California and Nevada and probably Panama in 1941.

*Trypanosoma vivax*—This is a very active trypanosome and was first discovered in the blood of cattle, sheep and goats in the Cameroons. Later it was found widely distributed throughout the tsetse fly areas of Africa. In addition to cattle, sheep and goats it also occurs in equines. Monkeys, dogs, guinea pigs and mice are not inoculable. It has also been found in various species of antelopes in the Belgian Congo. It can be distinguished from other pathogenic trypanosomes not only by its great activity but also by its morphological features. The bulk of the cytoplasm lies posterior to the nucleus giving to this part of the body which consists of a clear alveolar cytoplasm a swollen and broad appearance. The kinetoplast is at or near the posterior extremity and is well developed. The nucleus is central. Several species of tsetse fly are capable of transmitting *T. vivax* as *G. palpalis*, *L. oides*, *longipalpis* and *morsitans*. The development in the fly illustrates another type of evolution. In this instance there is no stomach phase of development, the multiplication of the trypanosomes taking place in the proboscis only. Crithidia forms are produced in the labial cavity. Later the hypopharynx is invaded and finally there are produced the infective metacyclic trypanosomes of the blood type. Mechanical transmission by means of *Stomoxys* has also been effected. *Trypanosoma unioformis* is a small form of *T. vivax* type.

*Trypanosoma lewisi*—Rats in many parts of the world show this infection which is rarely fatal to them. It is transmitted by the rat flea by a process of regurgitation. It can also be transmitted by the rat louse.

There are many species of trypanosomes in birds, frogs, fish, etc.

## HUMAN SPECIES

For some years, in a number of our text books of tropical medicine it has been stated that two varieties of African sleeping sickness occur in man and that these conditions follow infection with two species of trypanosomes the more virulent type of the disease, occurring in South Central Africa being due to *Trypanosoma rhodesiense* transmitted by *Glossina morsitans* and that of a less severe but more generally distributed type being due to *T. gambiense* and transmitted by *Glossina palpalis*. At present however, it appears that it is not possible to distinguish two distinct human trypanosomes transmitted by different species of tsetse flies.

*Trypanosoma gambiense* was discovered in man in 1902. In 1910 a trypanosome was found in the blood of a rat by Stephens and Fantham which had been inoculated with the blood from a human case of sleeping sickness in Rhodesia and to this parasite the specific name of *T. rhodesiense* was given. The trypanosome was recorded as a new species since it was noted that in the blood of laboratory animals in which the strain was kept alive a small percentage of the thick stumpy non flagellated trypanosomes

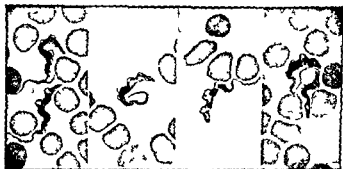


FIG. 34.—*Trypanosoma gambiense* (1d. presented by Professor F. C. Novy) (From Todd.)

had the nucleus situated posteriorly near the kinetoplast or even posterior to it. Also it was believed that the disease in man and in laboratory animals produced by this trypanosome ran a more acute course than was the case in disease produced by *Trypanosoma gambiense* and also that infection in man and animals was more resistant to arsenical treatment. From much study we now know that it is not possible to distinguish between these 2 strains of trypanosomes by the occurrence of posterior nuclear forms in animals. It may be emphasized that no distinction between the 2 strains based on the posterior nuclear character is possible from the direct examination of the infected person's blood. Moreover it has been shown that in some instances when *T. gambiense* is introduced into animals posterior nuclear trypanosomes occur. Also when *T. rhodesiense* is inoculated into the rat or guinea pig at times only a small proportion of the parasites will be seen to have their nuclei located posteriorly to the kinetoplast. Indeed there is some evidence to show that these posterior nuclear forms may result from the very rapid multiplication of the trypanosomes in the blood. Hence their number may vary with the virulence of the trypanosome or the susceptibility of the animal. From all the available evidence we can only conclude that sometimes *T. rhodesiense* is a more virulent strain of trypanosome than *T. gambiense*. The severity of the disease and its symptoms are obviously dependent not only on the virulence of the infecting parasite but may be affected by the severity of the infection and the susceptibility of the host.

A great deal of investigation has been performed to prove or disprove that *T. rhodesiense* is identical with *T. brucei* (of animals) which has been inoculated into man. When injected into rats *T. brucei* develops about the same proportion of posterior nucleated forms as *T. rhodesiense*. The proportion of such forms is usually small, about 5 per cent, but it varies with different strains. Many observers have suggested that *T. rhodesiense* is also identical with *T. brucei*, the common parasite of nagana of horses and cattle and of large game in Africa. Indeed Bruce, Kinghorn and Yorke and several other investigators regarded the two as identical. Apparently the great objection that has been made to this opinion is that *T. brucei* in wild animals and *Glossina* strains have a very much wider distribution than *T. rhodesiense* in man.

In this connection Taute and Huber were unable to infect 131 men with trypanosomes by inoculating them with the blood from 4 horses and 2 mules containing *T. brucei*. Yorke and his collaborators suggest that this apparent lack of pathogenicity for man may be due to individual immunity, since many normal sera exert a lytic action on *T. rhodesiense* to which *T. gambiense* is resistant. If such a natural immunity exists, some of the epidemiological facts are more easily understood. It seems not unlikely that the trypanosome of human sleeping sickness is probably the same species as that which infects certain animals (*T. brucei*, notably of wild game) but that it is a species which has become gradually accustomed to a new environment and finally specially adapted to life in the blood of man.

Different strains of *T. rhodesiense* may in some instances lose this power to infect man by passage through animals more or less susceptible, but in other instances retain it. Human beings differ in their resistance to strains of trypanosomes, as do other animals. Man is probably by the natural mode of infection immune to the trypanosomes of animals. It is perhaps only in those instances in which an individual especially susceptible becomes infected through a large number of virulent trypanosomes that the trypanosome becomes adapted to life in human blood and then may more frequently infect other human beings. While it is probable that some virulent human strains of trypanosomes are capable of infecting the majority of human beings, an animal strain, feebly pathogenic for man, will probably only infect at first at normally susceptible individuals, though later when such strains have become thoroughly established as parasites in man, epidemics of sleeping sickness may be caused by them. The differences in power of infecting of *Glossina* and of man and other animals of *T. gambiense*, *T. rhodesiense* and *T. brucei* may all be explained as changes which one species of trypanosome may undergo under different environment (Strong, 1934).

Corson (1939) concludes that *T. rhodesiense* is a parasite not only of man but also of wild and domestic animals and Duke (1939) states that the evidence at present indicates that *T. rhodesiense* is considerably less adapted biologically to man and his domestic animals than to the wild game.

Kleine regards *T. rhodesiense* as the form taken by *T. gambiense* when introduced into a new area and transmitted by tsetse flies of the *moritani* group. However, he thinks it is different from *T. brucei*. Other observers among them Duke regard *T. rhodesiense* and *T. brucei* as identical and Lavier believes that *T. gambiense*, *T. rhodesiense* and *T. brucei* are all one species. The conclusion reached by the International Commission on Human Trypanosomiasis was that *T. rhodesiense* represented *T. gambiense* transmitted by a different species of *Glossina*, namely *G. moritani*. However, Duke who examined the power of *Trypanosoma rhodesiense*, *T. gambiense* and *T. brucei* respectively to develop in *Glossina fuscipes* found that *T. rhodesiense* is as a general rule more readily transmitted by *Glossina fuscipes* than is *Trypanosoma gambiense* and this notwithstanding the fact that *Glossina fuscipes* is not considered to be the normal vector of *T. rhodesiense*.

Hoare (1938) found regarding the species of the *brucei* group (*T. brucei*, *T. rhodesiense* and *T. gambiense*) that they are indistinguishable from one another and indeed can hardly be regarded as distinct species. The size of the kinetoplast and its position (sub-terminal) was practically the same in all 3 species, though in *T. gambiense* the position of the kinetoplast was terminal in 8 per cent, whereas in *brucei* and *rhodesiense* it was terminal in 4 and 3 per cent respectively. (See table p. 172.) In view of these

facts. Some observers feel it appears unjustifiable to continue to regard these strains as separate species of human trypanosomes. Yorke (1942) gives additional evidence of this fact (*Trop Diseases Bull.* Sept. 1942).

**Trypanosoma gambiense**—This trypanosome occurs in the blood and lymphatic system and cerebro spinal fluid of man, domestic animals and game. Developing forms of the trypanosome are also found in *Glossina palpalis*, *G. morsitans* and other tsetse flies in the gut, other cecididia and metacyclic trypanosomes (occurring at the end of the cycle) are found in the salivary glands of the flies.

The flagellate has a thin slender curved fish shaped body the anterior end of which tapers to a fine point while the posterior end is relatively blunt. An undulating membrane extends almost the entire length of the body the margin of which is longer than the attached edge so that it is thrown into folds. In films stained by Giemsa's solution two chromatin staining areas are visible. The large trichonucleus staining reddish or reddish purple is central. Near the posterior end there is a deeply staining oval structure called the kinetoplast consisting of the blepharoplast and the parabasal body both of which stain red. In many preparations the distinction between the red dot like blepharoplast and the parabasal body cannot be seen. From the minute granule in this body (the blepharoplast or kinetoc nucleus) an axial filament or axoneme arises and extends anteriorly along the free margin of the undulating membrane and forward into a single whip like flagellum. Scattered granules of chromatin may be visible in the anterior part of the body. In fresh preparations an active writhing or lashing motility is observed. Progression is usually in the direction of the flagellated end but occasionally is in the opposite direction. The size of this trypanosome is variable. It is polymorphic some forms possessing a free flagellum and others without it. They vary in length from about 8 to 30  $\mu$ . When the parasites are numerous two different types may be observed (1) long and narrow and (2) thick stumpy forms. The former have a striking length of free flagellum. The latter show no free flagellum or only a very small portion of one. Many different forms intermediate between these 2 extreme types also occur. The short forms result at the time of the longitudinal division of the long ones and they may in turn develop into long types which also divide. In width they measure from 1 to 3  $\mu$ . It was formerly thought that the long slender forms and the short broad forms represented sexual differentiation. This is now known to be erroneous and Ohler and Prowazek obtained infection by inoculating a single trypanosome.

In the blood of domestic and wild animals the appearance of the trypanosome is the same but in some instances in a small percentage of the thick stumpy trypanosomes the nucleus may be found posteriorly sometimes near the kinetoplast or very rarely even posterior to this structure. This posterior nucleated form was formerly regarded as a distinguishing morphological characteristic of *T. rhodesiense*. Some authorities believe that only the metacyclic forms in the fly are infective for man. In man and animals the metacyclic trypanosomes introduced into the blood at the time of the biting of the fly undergo multiplication by longitudinal fission. They may enter either the lymphatics or the blood stream and usually increase sufficiently so as to give rise to febrile symptoms after a period of 10 days or more. In the blood all stages of division may be found when the parasites are numerous. Multiplication is by amitotic longitudinal fission beginning with the division of the blepharoplast and parabasal body and followed by that of the nucleus the undulating membrane and the body of the organism. It is believed that the axoneme and flagellum do not divide but that a new axoneme develops from the secondary blepharoplast and grows out becoming a new flagellum of the body of the new parasite.

**Cultivation**—The human trypanosomes of Africa have been grown in various modifications of the NNN medium by a number of observers. Rat blood or human blood is reported to be more favorable than that of the rabbit. However the writer has found cultivation of *T. gambiense*

difficult and in a number of instances has failed to secure a true growth. Although the trypanosomes were often kept alive upon this medium for several weeks subcultures could not be secured. Thomsen and Sinton (1938) have also emphasized the difficulties in cultivating *T. gambiense*. Developmental forms of the organism in cultures resemble those seen in the invertebrate host.

Christophers and Fulton (1938) in working on the respiratory metabolism of *T. rhodesiense* found that when the trypanosomes were deprived of glucose they rapidly became motionless and deformed and broken up and the uptake of oxygen ceased. Brutsaert and Hennard (1938) have recommended for the cultivation of trypanosomes a medium containing Ringers solution with NaCl Tyrode solution and citrated human blood. They also have reported good results with citrated human blood and Ringer's solution with cholesterol 0.5 gm per liter. And more recently by the addition of sodium p-lyanethol sulphonate (1 guide la Roche). Hawking has also used this method successfully. Cultures have also been obtained in chick embryos for eight generations by Longley *et al*.

**Transmission**—*Trypanosoma gambiense* is transmitted through the bite of flies of the genus *Glossina* in which has occurred a developmental cycle of the trypanosome. Under favorable conditions the fly after imbibing blood containing trypanosomes becomes infective in from 18 to 34 days in extreme instances not until 53 days. It then remains infective up to 188 days or for the rest of its life. In a few recent experiments under certain conditions of temperature and moisture the period of development in the fly is found to be as short as 12 days. Only a small proportion of the flies that feed on infected blood become infective (approximately 2 to 10 per cent). Infection is not transmitted to the pupa.

The cyclic development of *T. gambiense*, *T. rhodesiense* and *T. brucei* in the fly is similar and is peculiar to these types. After the fly ingests the infected blood the parasites accumulate and multiply in the middle and posterior portion of the gut. Usually between the 8th and 18th day long slender forms appear pass forward into the proventriculus and from there into the salivary glands and ducts. Here further development takes place into crithidial forms which attach themselves to the gland cells. From these are developed infective metacyclic trypanosomes (similar to the normal short type seen in vertebrate blood) which pass down the salivary ducts and through the channel in the hypopharynx from which they enter the bite wound.

Only these metacyclic trypanosomes have been regarded as infective for vertebrates; other forms in the digestive tract of the fly have not been found to produce infection. High temperatures 75 to 85 F are favorable for the development of the parasites in the fly while low temperatures 60 to 70 F are unfavorable for development but do not kill the ingested trypanosome. In Uganda West Nile District Brown *et al* and the infection rate in wild flies from 6-14% a high rate. In the same district three years later Gibbons found only flagellates in the gut in a few and in no case were the glands infected with trypanosomes. The mean index of transmissibility for the Congo is 3.63 but the metacyclic index is only 0.6.

Although the usual method of transmission it is believed occurs through the metacyclic phase the fly may transmit the infection mechanically at the time of its bite the infection resulting from the insertion of its proboscis moist with blood sucked from an infected individual within 2 or 3 hours previously. This method of transmission at times may be of great importance during epidemics when infected individuals

healthy people and the flies are all numerous in districts closely congested. Mechanical transmission of trypanosomiasis in horses by *Stomoxys* is a common method in the Far East.

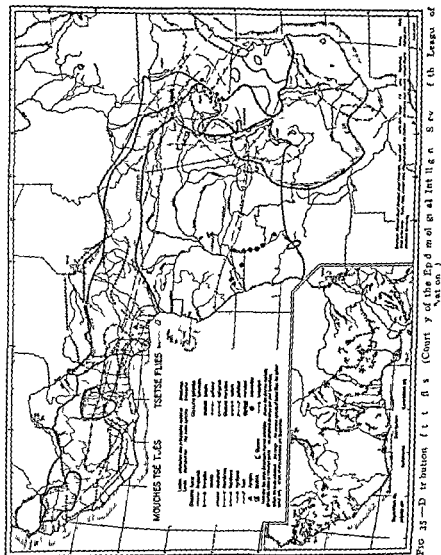


FIG 35--Distribution of the population of the League of Nations

The Epidemiological Service of the League of Nations states that

The results of the numerous researches effected up to 1931 into the habitat of the principal species of *Glossina* which transmit trypanosomiasis have been superimposed diagrammatically on the same map. Although this map only gives an incomplete picture of the local distribution of tsetse flies which depends on the immediate environmental conditions it nevertheless shows the areas which are liable to be infected.

**Glossina, the Tsetse Flies**—This genus is limited to tropical Africa and includes about twenty species several of which are of great medical importance because they transmit human trypanosomiasis (sleeping sickness)

The tsetse flies are brownish flies a little larger than the stable fly (*Stomoxys*) which they resemble. The proboscis projects forward horizontally and has a bulb at the base and a pointed tip. The palpi are long and form a sheath for the proboscis. On biting they rasp a hole through the skin with the pointed tip (labellum) and plunge the proboscis into the tissues.

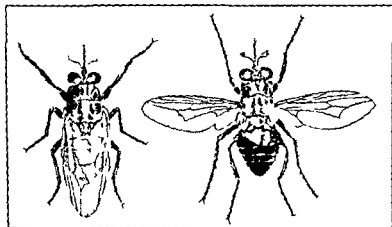


FIG. 36.—*Glossina palpalis*: in natural position and with wing outstretched (MacN. (after Doffin).)

In *Glossina* the arista of the antenna is plumose only on the upper side and the individual hairs are themselves feathered. The palpi are as long as the proboscis which they enclose as a sheath. The wings are carried flat, closed over one another like the blades of a pair of scissors and project beyond the abdomen. The most characteristic feature of the tsetse fly is the way the fourth longitudinal vein bends up abruptly to meet the mid-cross vein and then curves downward to run parallel with the third longitudinal vein before it turns forward again to end at the anterior border of the wing.

*Glossina* is peculiar also in the fact that the females are viviparous and deposit a single very large fully developed yellowish brown motile larva in shaded places in dry sandy soil. This burrows in to a depth of about 2 inches and immediately pupates. Moisture and sunlight are unfavorable for pupal development especially the latter so that pupae buried an inch deep and away from shade are killed. The period of gestation for *G. palpalis* is about 30 days and pupal development takes from 3 weeks (at 85°) to 12 weeks (70°). The adult flies live from 4 to 8 months. Their reproductive capacity is therefore very limited as compared with most diptera. They bite during the day even in bright sunlight and have even been known to bite beneath a mosquito net in bright moonlight. Both males and females bite and transmit the disease.

*Glossina palpalis* is the principal vector of *T. gambiense* and experimentally readily transmits *T. kinshipi* and *T. brucei*. It is a relatively large species with blackish brown abdomen and a grey thorax with indistinct brown markings. It is said to bite by preference crocodiles and other reptiles and the Situtunga antelope but it readily bites other game and domestic animals and man. It prefers brown or black skins to



white skins For range habits etc see section on prevention p 196 For reasons which are not understood in certain districts sleeping sickness does not occur although *G palpalis* is abundant

*G tachinoides* is a smaller darker fly showing distinct bands on the abdomen It is found in a belt along the southern border of the Sahara from the Atlantic to Arabia It resembles *G palpalis* in its habits and in its western range is a major vector of *T gambiense*

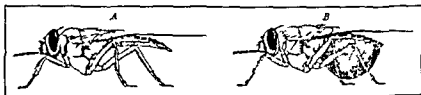


FIG 37—*Glossina morsitans* (A) before and (B) after feeding Lateral view (From Dofflein after Austin) (MacNeal)

*G morsitans* is somewhat smaller and lighter in color than *G palpalis* The abdomen is buff with dark cross bands which are interrupted in the midline It bites by preference large game or domestic animals and will bite man if these animals are not available It has a wider range than *G palpalis* and is not restricted to the immediate vicinity of water courses It is the principal vector of *T brucei* and *T rhodesiense*

Other proved vectors in which development of the parasite has been observed are Of *T gambiense* (experimentally) *G morsitans* *G pallidipes* (a natural transmitter in Uganda) *G brevipalpis* and *G fusca*

Of *T rhodesiense* *G swinnertonii* (in Tanganyika) and *G brevipalpis*

Of *T brucei* (experimentally) *G brevipalpis* *G pallidipes* *G palpalis* and *G tachinoides*

In certain regions *G tachinoides* was found more frequently naturally infected with *T gambiense* than was *G palpalis* (Lester 1936)

**Transmission by Stomoxys**—In certain areas where large numbers of cases of sleeping sickness are congregated even where *Glossina* does not abound infections may sometimes occur if other blood sucking Diptera as for example *Stomoxys* are present It is now well known that the trypanosomiasis of horses in the Philippines and elsewhere in the Far East caused by *Trypanosoma evansi* is usually transmitted by *Stomoxys* mechanically Duke, during 1934 has shown that *Trypanosoma rhodesiense* was readily transferred from an infected to a healthy monkey by the process of interrupted feeding of from 7 to 10 wild *Stomoxys* and that infection from antelope to antelope might also occur by *Stomoxys* However, transmission of trypanosomiasis by *Stomoxys* in man is probably relatively rare as sleeping sickness has shown no tendency to spread extensively in areas where *Glossina* does not abound

**Stomoxys calcitrans**—The stable fly resembles the common housefly in size and shape It can be easily distinguished by the black piercing proboscis extending beyond the head There are longitudinal stripes on the thorax and spots on the abdomen The proboscis on examination will be seen to be bent at an angle near its base The palps are short and slender The wings diverge widely The 4th longitudinal vein has a gentle forward curve The arista of the antenna is feathered on the dorsal side with simple straight hairs

The female lays about 60 banana shaped eggs in horse manure rotting straw or other decaying vegetation These hatch out in three days as larvae which turn into pupae in

two or three weeks. After about 10 days the fly emerges. The genus *Stomoxys* includes vicious biters. This is the fly which comes into houses before a rain and which has given the common housefly the reputation of biting before a rain.

*Haematobia irritans*, the horn fly, is a serious pest of cattle but rarely attacks man. It is about half as large as the stable fly. The palpi are much longer than in *Stomoxys*, being as long as the proboscis. They are thick and spatulate.

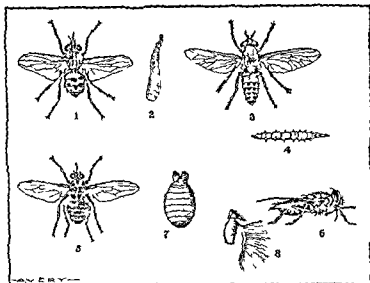


FIG. 38.—Illustrations in which the adult stage is important: (1) *Stomoxys calcitrans* (2) *Stomoxys calcitrans* larva (3) *Tabanus bryoni* (4) *Tabanus* larva (5) *Glossina palpalis* (6) *Glossina palpalis* developing (7) *Glossina palpalis* pupa (8) *Glossina palpalis* mandibles.

**Other Methods of Infection.**—Human trypanosomes may also be transmitted by coitus. The disease of horses known as dourine and due to *T. equiperdum* is commonly transmitted in this way. Koch noted the infection of 15 women in a fly-free district and considered infection to have come from their husbands who had contracted trypanosomiasis in a fly district and returned home. Bernard has also suggested this method of infection in prostitutes. While this method of infection in human beings therefore seems possible it is apparently rare.

Trypanosomiasis is also not usually transmitted hereditarily in human beings, although the parasites have been found in the placental blood of infected guinea pigs and rats and in the blood or liver of the embryos of such animals. Muhlens (1929) has reported a case of a child who was born in Germany of an infected mother who died 12 days after the birth of her child. The child itself subsequently became anaemic and suffered from periodic attacks of fever and died of trypanosomiasis some three months later. Schwetz (1935) has reported a case of trypanosomiasis in a baby of less than 20 days old. In this instance it was not possible to demonstrate trypanosomes in the blood of the parents and hence this was not an instance of hereditary infection. Indeed the evidence in regard to hereditary transmission of trypanosomiasis in human beings is to the effect that it is very rarely or not transmitted in this way.

Transmission of infection to children by the milk of infected mothers is another possible source of infection as it has been demonstrated to occur experimentally in animals and in a few instances trypanosomes have been found in the milk of women

**Reservoir Hosts**—Epidemics of sleeping sickness in man have occurred in which the trypanosome has been carried directly from man to man by the bite of the fly and sometimes mechanically. Recent evidence is to the effect that human beings are often probably the most important source of infection for the vector since it is now well recognized that in certain districts in which the disease prevails neither wild game nor suitable aquatic vectors exist which might serve as favorable reservoir hosts. In such areas the fly feeds for the most part on man and in such localities the rate of trypanosome infection of the fly has been found correspondingly high. In other regions however, it has long been recognized that wild game may serve as a reservoir of infection particularly species of antelope. Eleven species including bush buck reed buck and water buck have been experimentally infected by the bites of tsetse flies. The marsh inhabiting antelope sitatunga *Tragelaphus spekei*, is commonly infected under natural conditions and Duke found them infected in the islands of Victoria Nyanza four and a half years after the population had been removed in an attempt to eradicate an epidemic of the disease. Domestic animals in certain localities also constitute a reservoir of infection as *T. gambiense* has been found by various observers in cattle goats and sheep in Tanganyika Territory in East Africa. Many of these animals may carry the infection for long periods of time without symptoms of infection being apparent. The presence of nagana in cattle due to *T. brucei* in some localities constitutes a special reservoir of infection. Van Hoof (1938) has found that the native pig is an ideal reservoir of *T. gambiense* in the region of Leopoldville and Curasson has confirmed this fact in French West Africa.

*T. gambiense* can be transmitted also to most laboratory animals. In monkeys the infection is usually fatal but higher apes are more resistant and several investigators have found baboons immune to inoculations of infected blood. In rats mice guinea pigs and rabbits the infection runs a chronic course as it does in domestic animals. Wild game when infected usually exhibit no symptoms of disease although Corson who inoculated 8 antelope experimentally with *T. rhodesiense* found just before death or just after death trypanosomes in the cerebro spinal fluid. He suggests that the wild game in sleeping sickness areas have acquired a selective resistance from exposure to habitual fly bites with strains of *T. brucei* of low virulence. In some localities *T. rhodesiense* has been found to have no pathological effect on bush buck and reed buck but is pathogenic to oribi and sitatunga.

A number of instances of human individuals infected with trypanosomiasis and apparently in good health have been reported. Such individuals may also act as reservoirs of the parasite. There is no definite evidence of natural immunity from trypanosomiasis in human beings.

Age, sex, race and occupation bear no relation to susceptibility to infection but only exert an influence in connection with exposure to infection by *Glossina*.

## PATHOLOGY AND MORBID ANATOMY

The most striking lesions are observed in the lymphatic glands and in the central nervous system. The chief feature of the infection is a chronic inflammation of the lymphatic system due either to the mechanical action of the trypanosomes or possibly to their toxins from which there results an enlargement of the glands. Following this chronic

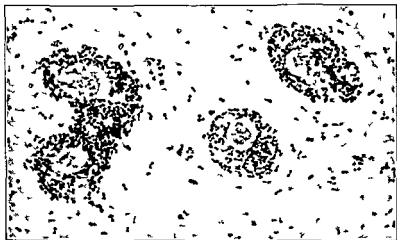


FIG. 39.—Section from brain of a patient with African trypanosomiasis showing perivascular infiltration of small round cells. (Section courtesy of B. C. C. well—Army Medical Museum Photo No. 46924.)

polyadenitis, a chronic inflammation of the lymphatics of the brain and spinal cord often takes place. The fever in the early stages is followed by meningo-encephalitis and meningo-myelitis, proliferation of the neuroglial elements and lymphocytes occurring and of the endothelial cells about the perivascular lymph spaces—particularly of the pia-arachnoid of both brain and cord. The process is most marked about the vessels of the pons and medulla. These two processes compress the vessels and lessen the blood supply to the brain and cord, which results in malnutrition, cerebral changes and the manifestations of a desire to sleep. The nerve cells other than those of the bulbar nuclei are but little affected. The trypanosomes are not confined to the vessels but are distributed in an irregular manner in the brain.

Macroscopically the points to be noted at autopsy are the adenitis in the neck, groin and other lymphatic glands and the changes in relation to the brain and cord. The cerebrospinal fluid is increased and often turbid; the dura mater may be adherent in places and the pia mater may show areas of thickening. There is usually congestion of the brain and the ventricular fluid is increased. The cord also shows evidences of congestion and there may be haemorrhages. The base of the brain may

be pale. The corda equina may be surrounded by gelatinous oedematous tissue. Sometimes there is ascites and pericardial fluid in excess. The lungs may show pneumonic changes. The spleen is usually somewhat enlarged.

However in some instances no gross lesions of the nerve centers or other organs are visible though usually on microscopical examination of sections evidences of a meningo encephalitis are detected with a varying amount of infiltration of lymphocytes, glia and endothelial cells in the perivascular lymphatic tissue of the brain cord and meninges. The changes are sometimes very similar to those seen in general paralysis of the insane. Sometimes small areas resembling granulomata are present (Durck's nodes) especially in the sections of the cortex. 'Mott cells' with fuchsinophile hyaline globules have been observed by Mott and others. Peruzzi believes that they are generally of neuroglia origin. Yorke, Wolbach and Stephenson have shown experimentally in animals that the lesions of the lymphatic or nervous tissues are due to an invasion

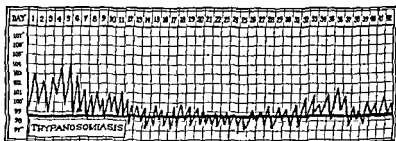


FIG. 40.—Typical temperature chart of trypanosome fever

by the trypanosomes. In the cerebral tissue, the frontal lobe, pons and medulla they were found in masses or nests.

Microscopical examination of the cerebrospinal fluid often reveals trypanosomes. Reichenow found that the cerebrospinal fluid does not become infected during the first 3 months after the beginning of the disease though he has never seen a case of more than a year in which the cerebrospinal fluid did not contain trypanosomes. Trypanosomes may be found in sections in the intercellular spaces in the brain and in earlier cases of infection they may be present in the lymph channels throughout the body. They are found particularly in the blood, lymphatic glands and lymphatics during the febrile periods of the disease and in the tissues and cerebrospinal fluid in the cases with nervous symptoms.

Scott (1938) has reported a case of a native child 14 years of age in whom *T. rhodesiense* was found in the blood during life and the patient was suffering from ascites and enlarged glands of the neck. In spite of treatment the child died two months later and enormous numbers of trypanosomes were found in the ascitic fluid.

Peruzzi has found in experimental infections of monkeys that myocarditis of a severe nature may be present and that this is due to collec

tions of trypanosomes in the muscle cells. This myocarditis was always associated with exudative pericarditis.

### SYMPTOMATOLOGY

The disease may be divided into 3 stages: (1) incubation, (2) febrile or glandular, and (3) cerebral. The clinical manifestations are irregular both in intensity and in their duration. The incubation period may be as

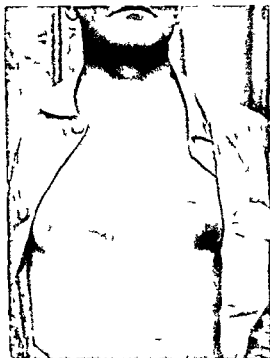


FIG. 41.—Rash of human trypanosomiasis. (Photo R. M. Kay.) By permission from Manson & Tropical Diseases.

short as 10 or 12 days from the time the patient was bitten by the tsetse fly to the time when trypanosomes may be found in the blood. In other cases the incubation period may be 2 or 3 weeks. Duke (1938) has reported a case of a volunteer whose blood showed no trypanosomes until the 51st day after his first inoculation of infected blood and the 18th day after the second inoculation of blood. Nevertheless his blood was infective to a monkey 20 days after his first inoculation. In a third group of cases the patients may carry the trypanosomes from two to five years before showing symptoms. Seven of Koch's African porters showed trypanosomes but continued to do heavy work. Naturally infected animals are able to perform work for long periods of time without symp

toms Following the bite of the tsetse fly, there may be induced more or less local inflammation about the point of the bite. This usually subsides within 48 to 72 hours. All but one of Carson's volunteers showed a characteristic local reaction at the site of the infecting bite. Duke (1939) states that of 17 volunteers infected 5 showed local reactions and 12 no reaction at all. A local reaction has been said to be more marked in the case of the more virulent strain *T. rhodesiense*.

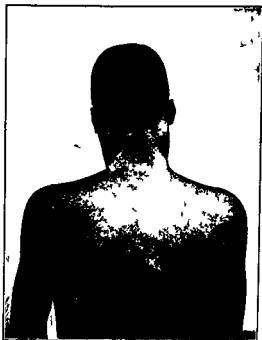


FIG. 42.—Swelling of the glands of the posterior cervical triangle. Winterbottom's sign (Aft & K. ch.)

After some 14 to 21 days or longer, following the bite fever may occur. The natives may show no fever before the period of sleeping sickness but in Europeans the primary fever is almost always present. The temperature curve is usually of a markedly remittent type, approaching normal in the morning and rising to 103° or 104°F or higher in the evening. The fever may disappear and recur at irregular intervals. The pulse rate and respirations are increased. The spleen and liver may enlarge and it is often difficult to exclude malaria except by blood examination. Early in the disease there may be evidences of involvement of the nervous system: neuralgic pains and headaches are common. There may be insomnia and difficulty of concentration for mental work.

Erythematous eruptions consisting of pinkish patches, irregular in position and outline, usually round or oval in shape with a clear center may appear particularly on the trunk or thighs (Fig. 41). Dryness of the

skin is rather constant and pruritus is often present. A mild bluish appearance of the trunk is also said by Masters to be very characteristic in natives. Kellesberger has noted a loss of the shiny, oily character of the skin. There may be patches of painful local oedema about the hands or feet and oedematous swellings about the eyes or joints (Fig. 43). Many of these cutaneous phenomena last for a few days and reappear in other areas. The enlargement of the lymphatic glands, especially



FIG. 43.—Cases of trypano omiasis showing the o d ma espe ally about eye (Aft r Koch.)

those of the posterior triangle of the neck. Winterbottom's sign is very important in diagnosis (Fig. 42). The glands vary in size from that of a split pea to that of an almond, rarely larger. They are at first soft and elastic but later become hard and fibrous. The supraclavicular, epitrochlear, axillary and inguinal glands may also be swollen. They are usually discrete. Upon puncture they may show trypanosomes when the blood fails to show them. Deep hyperesthesia, which is known as Kerandel's sign (after a physician who contracted the disease and described it) is sometimes noticed. The slightest pressure feels like a



bruise and is exceedingly painful. The sensation of pain is sometimes slightly delayed. A puncture with a hypodermic needle is said to feel like a red hot poker and an accidental knock by a walking stick gives excruciating pain. As the disease progresses there are usually asthenia and anaemia with considerable weakness. No wasting of the body however, may be present until later stages. Lesions of the eyes have sometimes been reported, such as conjunctivitis, iritis, keratitis, retinal changes and deep oedema of the lower eyelids. Manson Bahr has observed a toxic irido cyclitis and choroiditis. In many epidemics however lesions of the eyes have not been reported. The appetite is usually good until the sleeping sickness stage. The febrile stage may go on for years or it may either end spontaneously or be cured by treatment.

There are great variations in the severity of the disease. Sometimes in Europeans the symptoms of disease may be very transient and slight. Lester (1939) emphasizes the mild character of the disease in Nigeria and that the proportion of severe nervous cases in Uganda, the Congo and the Sudan does not appear to be any higher than in Nigeria. He found that in West Africa only about 5 per cent of the patients developed severe nervous manifestations.

Kellersberger divides the cases clinically into three groups. First, those in apparently good health but in which trypanosomes may be found in the blood stream in about 4 per cent of the cases. It apparently usually takes several weeks for the organism to multiply sufficiently to be found in the blood by ordinary examinations. Several months after this the lymphatic glands become inflamed and enlarged and the trypanosomes may also be found by gland puncture. These cases usually have some remittent fever. The second group of patients show definite clinical symptoms. In addition they often go to sleep in the daytime and have nervous symptoms. The second group comprises about 75 per cent. The third group of advanced cases constitutes about 13 per cent and the mortality among them is high.

*The Spinal Fluid*—In the early stages of sleeping sickness lumbar puncture may reveal a normal fluid but in the later stages of the disease with nervous symptoms there is usually increased pressure. The liquid is more or less opaque and there is a positive globulin reaction determined by Pandy's phenol test. Leucocytes may range from zero to a thousand per cubic millimeter. Centrifuging of the fluid frequently reveals parasites. In advanced cases trypanosomes may also be more numerous in the blood. In unfavorable cases the so called mulberry or muriform cell as described by Pearce may be present in the spinal fluid and the globulin reaction become more strongly positive.

*The Cerebral Stage*—The first signs of this may be tremor of the tongue and fingers, headaches, delusions, hysteria and mania. Oedema of almost any part of the body may occur. Only the first stage of the disease is usually curable. If the cerebral stage is reached and the trypanosomes appear in the cerebrospinal fluid death is frequently inevitable. This stage lasts from a few weeks in acute cases to a few

months or even longer in chronic ones. A few patients have lived for years and died from some intercurrent disease.

In the beginning of this stage changes in the habits of the patients are apt to occur, the disposition often being modified for the worse. They become apathetic and dull, and there is disinclination for exertion. Many find it difficult to walk, and even forget to masticate their food. Fine tremors of the tongue, hands, arms, legs, and even of the abdomen appear. The gait is apt to become peculiar, as though there were difficulty in raising the feet from the ground, the patient shuffling along or throwing his feet upward in walking. There is no paralysis as a rule, and the superficial reflexes are normal, but the deep reflexes are first increased and then lost. The speech becomes low and tremulous, like that of a tired, sleepy individual. Delusions and mania are fairly common. Romberg's sign is sometimes present. Argyll Robertson pupil has not been noted. Later there may be rigidity of the neck and legs, and a tendency to permanent flexure of the legs on the thighs and abdomen. The fever in this stage is very variable. There may be daily fever, with the temperature subnormal in the morning, or there may be no fever at all, and the temperature subnormal. The pulse is usually accelerated, 90 to 140, and out of proportion to the temperature. There may be Cheyne Stokes breathing before death, and congestion and oedema of the lungs with pneumonic patches are common. The patient often shows a tendency to sleep, or to lie in a lethargic condition, even in the bright sunlight. The alkalinity of the blood has been reported as diminished, and it has been suggested that this is probably due to amino acids secreted by trypanosomes and produced by the action of the amino acids upon the serum proteins.

As the disease progresses emaciation becomes common, the tension of the pulse low, and the systolic blood pressure extremely low. Muscular weakness is very marked, the tremors are pronounced, saliva dribbles from the mouth, and the urine and faeces may be passed involuntarily. Bedsores form, the pulse cannot be felt at the wrist, the temperature becomes subnormal, coma sets in, and death follows.

Of complications pneumonia is very common. Laryngitis and oedema of the glottis have been reported. Epileptiform symptoms are common. Castellani and Chalmers point out that the commonest complication during the last stage is a cerebrospinal meningitis due to streptococci, the pneumococcus, or the meningococcus.

Cases in Central African natives are frequently complicated by the presence of other diseases, such as malaria, ankylostomiasis, and schistosomiasis. The emaciation which is so common in the later stages of the disease also may be due largely to starvation and sheer neglect. Many of the cases die of a terminal pneumonia or a dysenteric infection.

**Blood Changes.**—In some cases there is a gradual diminution of the red blood corpuscles and haemoglobin. Normoblasts are sometimes then present. The leucocytes are normal in number, as a rule, but there is generally an increase in both large and small mononuclear cells. The former may constitute from 20 to 30 per cent, and the latter from

bruise and is exceedingly painful. The sensation of pain is sometimes slightly delayed. A puncture with a hypodermic needle is said to feel like a red hot poker and an accidental knock by a walking stick gives excruciating pain. As the disease progresses there are usually asthenia and anaemia with considerable weakness. No wasting of the body, however, may be present until later stages. Lesions of the eyes have sometimes been reported, such as conjunctivitis, iritis, keratitis, retinal changes and deep oedema of the lower eyelids. Manson Bahr has observed a toxic irido-cyclitis and choroiditis. In many epidemics, however, lesions of the eyes have not been reported. The appetite is usually good until the sleeping sickness stage. The febrile stage may go on for years or it may either end spontaneously or be cured by treatment.

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glandular enlargement is more marked at the beginning of the disease than in later stages

**The Skin**—Erythematous areas may be present in Caucasians and in Africans but in the latter are often difficult to detect. Localized oedemas are rather marked features. The skin may be very dry and itch markedly.

**Other Manifestations**—The spleen may be enlarged, the respirations may be more rapid than normal and the blood show a secondary anaemia. In a blood examination the large mononuclears often show an increase with a normal white count. The eye may show keratitis or irido cyclitis in trypanosomiasis. Trypanosomiasis seems to favor abortion and still births in this respect resembling syphilis.

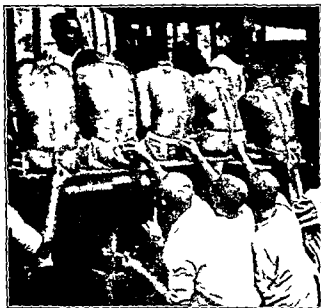


FIG. 44.—Lumba pun tur f r d agnos of sl p ng kness. The n tv ho p t l att nd nts coll ct the li q u d the p n t r was m de b f r h nd by th do to. (After Cl. Jamot from Joyeu.)

### DIAGNOSIS

The finding of the trypanosome is conclusive. It should be borne in mind that the glandular enlargements with the erythema and headache may suggest syphilis. Another source of confusion is the positive Wassermann test often obtained in sleeping sickness. There may be an increase in the large mononuclear cells which is also seen in malaria, kala azar and syphilis so that such findings are of little assistance in differentiation. For the discovery of the trypanosomes examinations should first be made of the peripheral blood in fresh or stained films. In a small percentage of the cases the parasites are fairly numerous but in the great majority

30 to 40 per cent of the leucocytes. Sometimes there is a terminal increase in the polymorphonuclear leucocytes before death. The chemistry of the blood has been studied by Unna and Tielman and also by Takinoff and Nierenstein who have shown that in animal trypanosomiasis the acid of the plasma is increased. Christophers and Fulton (1938) in working on the respiratory metabolism of *T. rhodesiense* found that the most striking feature was the utilization of glucose in the formation of acid products and the large oxygen uptake. When the trypanosomes were deprived of glucose, they became rapidly motionless and lysed and the uptake of oxygen ceased.

The phenomenon known as auto-agglutination is often noticeable in fresh preparations even when the specimen has been made with care. The red cells are clumped in masses and do not form rouleaux. Macfie and Johnston have called attention to the phenomenon of auto-erythrophagocytosis which is occasionally observed. Blood changes are further referred to under 'Diagnosis'. The urine shows no important pathological change.

#### THE SYMPTOMS IN DETAIL

**The Nervous System**—Headache and lack of mental concentration may be early features of the disease. Deep hyperaesthesia, or Kérandel's sign, is often present. Patients tend to be morose and apathetic. Tremor of tongue and lips are rather constant signs about the commencement of the stage of sleeping sickness. Early insomnia gives way to the drowsiness that characterizes the second stage. There is very little disturbance of sensory or motor functions until near the end. Epileptiform convulsions may be late manifestations. Coma deepens as the end approaches.

**The Temperature Curve**—The febrile paroxysms, which may not be present in natives until the sleeping sickness stage, show great irregularity of course and a marked remission in the morning. The fever may be absent for several weeks to return later. Trypanosomes are more apt to be present in the peripheral circulation during the fever than when the temperature is normal.

**The Circulatory System**—The pulse tends to run from 60 to 120 beats per minute and is fast even without fever. In the cerebral stage it may reach 140. The tension is low and the systolic pressure tends to be extremely low during the later stages of the disease.

**The Lymphatic System**—Most important in diagnosis is the enlargement of the lymphatic glands, especially those of the posterior cervical triangle (Winterbottom's sign). Other enlarged glands may be the supraclavicular, epitrochlear and axillary glands. The inguinal glands suffer enlargements so frequently as the results of wounds and infections of the feet that their enlargement is of less diagnostic value. The natives of certain parts of Africa not only attach great diagnostic importance to gland enlargement but it is said they imagine they cure the disease by removing the glands with various primitive cutting tools. The glands are not painful, do not become matted together and rarely suppurate. The

In the stage of sleeping sickness and in cases with early nervous symptoms trypanosomes can often be demonstrated in the cerebrospinal fluid. It is advisable to centrifuge and to examine the deposit. There is an increase in the globulin content and in the cell count in which the type of cell depends upon the chronicity of the infection. In especially unfavorable cases the so called mulberry or muriform cell has been described by Louise Pearce and the globulin reaction (Pandy's test) is strongly positive.

If the trypanosomes cannot be found in smears a rat guinea pig or monkey may be inoculated with the material. Feebly virulent strains may produce only a mild infection but examination of the blood at intervals will usually reveal the trypanosomes.

When the organism cannot be demonstrated the following tests of the patient's serum have been used as aids in diagnosis. (1) The formol gel test described in the section on Leishmaniasis is said to be positive in outspoken cases (Morrison and Dye). Hope Gill (1938) believes the reaction of value if it is positive within 60 minutes. (2) Autoagglutination of the red cells is often marked and constant and suggestive of trypanosome infection. In a fresh blood preparation the red cells become aggregated into large clumps which may be visible with the naked eye. They are not rouleaux. The reaction is not specific for trypanosomiasis however and is not of much diagnostic value. (3) Trypanolysis may be demonstrated by mixing unheated (fresh) serum with a suspension of trypanosomes and incubating for an hour. The reaction is said to be positive in a majority of the cases. (4) The adhesion phenomenon—Brown and Brown (1933) believe the red cell adhesion test is a specific serological reaction. It consists of incubating together trypanosomes, the immune serum, complement and the human red blood corpuscles. If the serum is homologous the red cells become firmly adherent to the trypanosomes. The reaction has been used in epidemiological studies in wild animals. The practical value of the latter procedures is often restricted by the technical difficulties in securing suitable suspensions of trypanosomes.

In the case of dourine of horses the complement fixation test has been employed for diagnosis by the Taliaferros (1934), Kelser (1936) and Koch (1939).

Guilbert Jospin and Thiroux all have advised examination of the bone marrow for diagnosis. Guilbert found the bone marrow puncture was positive in 18 out of 20 cases. However van den Branden (1938) in a study of the bone marrow of 5 rats infected with *T. brucei* found the trypanosomes in the blood of all the animals but only in 2 instances were they present in the bone marrow. Linhard found that in man sternal puncture with a lumbar needle revealed trypanosomes more frequently than peripheral blood examination the ratio being 211:129.

### PROPHYLAXIS

The most important measures under prophylaxis include

- (1) The diagnosis, isolation and chemo-inoculative treatment of those infected and prophylactic inoculations of healthy individuals.
- (2) the inspection and control of individuals coming from infected into uninfected localities.
- (3) the protection of man from bites of *Glossina*.
- (4) removal of the inhabitants from badly infected regions and the avoidance of localities in which large numbers of natives are infected or where the tsetse flies show a high rate of infection.
- (5) destruction of *Glossina* and their breeding places.
- (6) the destruction of possible animal reservoirs of the trypanosome.

they are extraordinarily scanty and may not be discovered in spite of repeated and prolonged examination. Kellersberger in a report of 9000 cases states that the trypanosome was demonstrated in the blood in only 4 per cent of the early cases. Often the most successful procedure is to examine the fresh preparation with a low power objective and search first for movement among the red blood corpuscles such movement suggesting the presence of trypanosomes. Thick films of the blood may be treated with Giemsa's stain and examined with the oil immersion lens. Broden and others have recommended examination of citrated blood (9 cc of blood to 1 cc of 6 per cent sodium citrate solution) centrifugalizing it twice at 1000 and at 1500 revolutions for 10 minutes.



FIG. 45.—A dying case of trypanosomiasis. (Courtesy of E. R. Kellersberger.)

The supernatant fluid and leucocyte cream are removed and centrifugalized at a higher speed for 20 minutes. Smears made from the sediment are then examined for trypanosomes.

Smears from the gland juice may show the trypanosomes when they cannot be found in the blood. The material is obtained by puncturing an enlarged gland. The syringe and needle used must be dry since water distorts the parasites. In the study of 32 cases, Kleine found 24 had trypanosomes in the glands and blood. Four had them in the glands but not in the blood and 4 had them in the blood only.

In a diagnostic study of 336 cases, Broden obtained 87 per cent of positives from gland puncture, 80 per cent from centrifugalizing the supernatant fluid left from the second centrifugation of the blood and 45 per cent from the spinal fluid examinations.

However he emphasizes that there can be little hope of eradicating the disease altogether by these measures

**Chemo prophylaxis**—In earlier years it was suggested that the drug Bayer 205 might be of value as an efficient prophylactic. However, it has been found that a prophylactic injection of Bayer 205 does not always prevent actual infection. Fourché in the Congo has reported that the injection of one gram in the case of adults and 0.3 to 0.75 gram in the case of children by the intravenous route had a definite prophylactic value during seven months' observation. Van den Branden has also employed this method of chemo prophylaxis and inoculated all the inhabitants of one village. Six months later only one case of sleeping sickness was found. Duke found experimentally that an injection of one gram of Bayer 205 will protect against *T. gambiense* or *T. rhodesiense* for at least 3 months. Between 70 and 80 native volunteers were used in the experiments during several years. There have been no accidents and it has been suggested that further use of this drug should be made for prophylaxis.

**Inspection of Travellers**—Tsetse flies often settle on the backs of pedestrians or of motor cyclists and enter the hoods of motor cars. Thus infected flies may frequently be carried long distances. Jack has reported that a pedestrian in some instances has carried flies for 10 miles and that in the hoods of motor cars they have been transported as much as 50 miles in a day. When the moving object stops the fly frequently leaves and seeks shaded areas in the vicinity. For these reasons the control of traffic on trails and roads leaving badly infected fly areas has been recommended and arrangements have been made to rid individuals, animals and conveyances of any accompanying tsetse flies through the use of petroleum or pyrethrum sprays. In some localities suitable screened chambers have been constructed into which motors and other vehicles may enter and the flies destroyed within them. Such measures have been carried out particularly in Southern Rhodesia. Procedures of this nature applied along the borders of the tsetse infected zones may prevent infected flies from reaching adjoining areas which are free from infection. Unfortunately no satisfactory repellants against tsetse flies are known. In French West Africa and parts of the Congo there is control of the movements of the natives in all sleeping sickness districts and none are allowed to travel except with a ticket obtained at a hospital after examination showing that they are free of the disease.\*

**Protection from Glossina and Removal of Inhabitants**—The screening of infected individuals until the trypanosomes disappear from their blood through treatment is desirable in regions where infected flies are prevalent. In some regions protective measures involving especially a reduction of the man fly contact by methods of communal clearing and movement of the population and its concentration in healthier areas has proved the most hopeful line of attack against the spread of the disease. In certain badly infected areas the evacuation of the population from the scattered villages in fly infected woodlands and the establishment of them in large clearings where tsetse cannot live has sometimes

C. could reports a considerable number of people apparently in perfect health are found to be infected with trypanosomes on the occasion of a visit to the Pasteur Institute, Brazzaville (Free French Africa) to obtain a health passport. The disease may show period of latency or silence lasting many years. *Trop Dis Bull*



In some localities, the isolation and proper treatment of all infected individuals has greatly reduced the amount of the disease. Thus in the Belgian Congo it is reported that more than 3 million people are examined annually, all positive cases being recorded and persuaded to attend regularly at one of the numerous treatment centers. For the period 1930-34 there was noted a decrease of new cases to about 50 per cent. Actual figures reported are given in the following table.

	Natives examined	New cases	Percentage of new cases to natives examined
1930	2 779 448	35 562	1.2
1934	3 824 097	24 010	0.63

In the Cameroons where sleeping sickness has increased steadily during the past ten years Millous (1935) reports that during the year 1933-34 546 000 natives were examined, of whom 61 800 had the disease and only 3 300 of these remained carriers of trypanosomes after treatment. In the lower Congo the *Foreams*, an organization interested in anti sleeping sickness work, has caused a systematic study of the inhabitants to be made for trypanosomiasis since 1931, all cases found infected being treated. In 1934 more than 38 000 lumbar injections were given and it is reported that the prevalence of the disease has been reduced from 2.45 per cent in 1931 to 0.9, per cent in 1934. It is stated that in some localities the natives now have such faith in the successful cure of sleeping sickness as they have in that of yaws and that this renders treatment comparatively easy. In Nigeria, a somewhat similar system of inspection and treatment has also been carried out. The organization for such work includes a survey party which makes a census of the area in question, examines the glands of each inhabitant, makes blood slides of suspected cases and notes all individuals in whom trypanosomes are demonstrated. A treatment party follows and injections of either Bayer 205 or trypanamide are given in the positive cases.

MacQueen (1936) believes that in Nigeria treatment alone cannot control the disease, and he insists that protective measures such as communal clearing and the movement and concentration of populations can be the only radical cure. Lester (1939) points out that in Nigeria repeated surveys among the natives (in the Muslim Emirates) are not popular and that the compulsion necessary for them is in many localities undesirable. Also the cost of these continual surveys is prohibitive, for it is estimated that there are nearly a million cases in the northern provinces of Nigeria alone, and hence the treatment of that number every year would be impossible. Nevertheless Lester writes that recent work has shown that mass treatment followed by the establishment of sleeping sickness dispensaries, detection and treatment of new cases, has reduced the infection rate to between a quarter and a tenth of the old figure.

the banks of adjacent streams. The tsetse fly especially haunts the underbrush for some 10 to 15 yards along the bank of streams where the females deposit their larvae. The insects rarely extend their feeding distance for more than some 15 yards, whether on the land side or on the water side. However they often follow with great persistency an individual who has just passed through this narrow belt frequently for a mile or more. Deep shade is essential for the development of the flies. Hence it has been found that clearing out of the underbrush for a distance of some 30 yards on either side of the water courses was usually an effective measure. It is important to clear the brush not only around the villages but about fords and boat landings where otherwise flies are plentiful.

In a section of the Southern Rhodesian Portuguese border the invasion of tsetse was so great formerly that a number of the farms had to be vacated on account of the heavy mortality of cattle from trypanosomiasis. Forest clearing along the border was undertaken in 1932. The clearing which varied in width from some 50 yards up to about a mile had been extended by 1934 for a length of 35 miles. Indications up to the present show that it has been remarkably effective. Trypanosomiasis has died down nearly to the vanishing point on the Rhodesian border and the evacuated farms have been reoccupied and cattle are being raised upon them.

Clearing of the underbrush along water courses has been particularly effective in parts of Northern Nigeria and in Tanganyika. In Southern Nigeria the possibility of control of the fly by modifying vegetation is more difficult. The rate of growth is so great that it is almost impossible to keep any large area in the rain forest belt thoroughly clear of vegetation. Also while brush clearing is very effective with reference to *G. palpalis* and *G. tachinoides* it is often not effective against *G. morsitans* as this species will frequently cross carefully prepared clearings over a mile in width. In the Gold Coast Stuart (1937) and in Tanganyika Nash and Jackson (1938) have shown that tsetse occurs along the rivers except in the rainy season. Then it migrates into the dry country. In the Gold Coast belts of land half a mile wide are cleared along the water courses and the brush wood burned over the stumps of trees which are most capable of rapid regeneration. Excellent results have been reported in rendering the areas fly free.

Experimental evidence reported by Swynnerton (1936) shows that sight guides flies to their moving host and that the road to be safeguarded from some species of the flies from adjacent bush must have at least 200 and preferably 500 yards on either side cleared of fly cover. Scent also seems to guide flies to their favorite host.

**Burning**—Some entomologists believe that organized grass fires have proved very valuable in reducing the incidence of flies in many districts. However the method has its limitations since continuity of the grass must be adequate for successful burning. It has been employed in large areas in Tanganyika and Southern Uganda. Fires are permanently effective only if they can be carried up to and across barriers

proved of value. Obviously where this principle is carried out suitable land for farming requirements must be provided. Rapid clearing of the bush in the new settlements to make them fly free is necessary. In a number of instances the restriction and settlement of the population in areas which later proved unsuitable has caused more damage through poor nutrition due to lack of food supply and famine than sleeping sickness would have done. However in parts of Uganda and the Sudan and in Tanganyika where the populations of the old, badly infected endemic regions have been moved to less dangerous regions the disease has been brought under control. In such districts the people have been taught to build modern compounds and to lay out their villages in a sanitary manner. In Uganda, in an attempt to stop an epidemic of the disease and with the hope that the tsetse flies in the region would eventually cease to be infective the Government moved the entire population of the Sesse Islands and of the neighboring shores of Victoria Nyanza to fly free areas in the interior. Three years after the depopulation of the district local flies were captured and were still found to be infective and capable of conveying the disease to laboratory animals. Apparently the *Sitatunga* antelope naturally infected in the region had served as a continual reservoir of the infection for the flies.

Methods of control by the reclamation of areas which have become overrun by the fly also have been recently attempted, particularly in Tanganyika by Swinnerton in Southern Rhodesia by Jack and in Nigeria by Nash. Flies can generally be eradicated by clearing the vegetation with the axe but this method may have serious drawbacks in many localities in connection with the problems of erosion. It usually is expensive also.

Abolition of contact between man and the fly is obviously of great importance. In endemic regions the fly areas should be avoided as far as possible and it is recommended if such regions have to be traversed that the journey should be made during the night when tsetse flies do not bite. Also in areas in which the natives and flies are infected infected individuals should be isolated under mosquito nets or in fly proof houses. The movements of infected individuals must also be prevented as far as possible. It has been suggested that examination of the cervical glands should be generally employed in eliminating infected individuals and in preventing them from traveling. Years ago it is said the slave dealers employed such a method to protect themselves from buying diseased individuals. However since the cervical glands are often enlarged in other pathological conditions this method of diagnosis is often not of value. Also in many cases of trypanosomiasis the glands are not appreciably enlarged. Even when the microscopic examination of the blood is made one may fail to find the trypanosome and an infected individual may pass undetected.

**Measures Directed against *Glossina***—One of the most efficient preventive measures directed against the flies and in eradicating them from native villages has been the clearing of the underbrush from along

the optimum at which the flies live longer and breed more rapidly than in drier or in moister air. A relative humidity of 65 per cent was unfavorable and in moister air the flies nearly always refused to feed and were found to die off very rapidly. The reason why high humidities were unfavorable is still obscure. Flies were found to metabolize fat most rapidly in dry air and presumably to produce metabolic water to compensate for excessive evaporation. In general it has been found that when the humidity is high the *Glossina* are scarce and that the flies do not breed so well in such an atmosphere. Johnson and Lloyd found that in the rainy season (May to October) only 20 to 40 per cent of female *Glossina tachinoides* were pregnant but in the dry season (November to April) 60 to 80 per cent were pregnant.

Gibbons in the West Nile District Uganda (1941) found palpalis active at temperatures at 70 F (21 C) to 85 F. Most active at temperatures about 80 F (26.6 C).

Nash (1937) has shown that the maximum shade temperature which *G. morsitans* and *G. tachinoides* can stand is 41 C (106 F) and since this temperature is often exceeded in the woodland in the drier parts of Nigeria the fly must resort to the shade of dense vegetation along streams in order to survive during a large part of the dry season. In Tanganyika however it need never leave the woodland to survive. Nevertheless at certain times of the year the surface temperature of the soil is so nearly lethal to pupae that partial brush clearing would probably be sufficient to eradicate the fly. Nash has demonstrated this in Tanganyika by exposing pupae in the brush at different depths in the soil.

Jackson (1934) found that during the dry season in Tanganyika *Glossina morsitans* increases in numbers in the drainage valleys as distinct from the bordering woodlands. Observations by him and by Burtt support the contention that the increase of the fly in the drainage valleys in the hot dry months is due not to a search for better shade conditions but to the fact that these areas constitute a feeding ground and that the fly must visit them more frequently at this season when the onset of hunger is hastened by hot dry conditions.

Nothing of real value is yet known in regard to biological control of the flies. Lamborn attempted to introduce a predatory insect which would destroy the pupae of *Glossina* but without definite success. Some years ago Austen suggested the introduction of the chalcidid *Spalangia* into areas where *G. morsitans* prevailed. One species *Sytemophyrum glossinae* was reared in the laboratory and distributed over an area of Lake Nyassa. In the course of 3 months it was found that some 8 per cent of *Glossina* pupae had become parasitized. Apparently no practical use has been made of such methods of biological control in recent years.

**Destruction of Game**—Yorke and Duke (1936) and others have suggested that in the areas where *G. morsitans* breeds all game animals near human habitations should be killed in order to limit the problem of fly food to human blood sources with the idea that if the human beings were then protected there would be as a result a rapid reduction in the fly population. Attempts to starve or drive out the flies especially *G. morsitans* in wide areas by destruction of wild game has been tried in several places but with the exception of the more favorable reports in Southern Rhodesia such methods have proved either impossible or undesirable. Bevin (1939) records that during recent years a hundred thousand pounds has been spent in Tanganyika in the endeavor to control the fly by the destruction of game and hundreds of thousands of

impassable to the fly. Such barriers have been provided in parts of Tanganyika by clearing broad bands of vegetation, the country thus being divided into blocks, in each of which the fly can be attacked without risk of reinfestation. On the other hand, other experiments in Tanganyika have shown that if blocks of tsetse infected bush are protected from fires for several seasons the growth becomes so dense as to be highly unfavorable to certain species of fly. In one block of 4 square miles which was protected from fire for 3 years the number of *G. swynnertoni* were reduced by nearly 70 per cent although the game in the area increased slowly in the same period. At the same time in a second block where grass burning proceeded normally the flies increased by over 300 per cent.

In Tanganyika normally the bush is burned each year during the latter half of the dry season. It has been found that when annual burning is stopped the fly population decreases. Lester says that this effect starts at the end of the first dry season as soon as fire is excluded. The number of flies fails to increase in the normal way at the start of the rains. How fire exclusion acts on the fly population is not clear. Whether the decrease is due to hunger, from poor visibility, or movements of game, or to an increase of small ants or other pupal predators or to increase in soil or atmospheric humidity is not known. Before fire exclusion can have any practical application a number of these questions must be answered. Also it is not yet known how many years of fire protection would be required to bring the fly population down to nil or negligible figures.

**Traps**—As a subsidiary measure, many attempts have been made in the wholesale catching of the fly by traps. A trap devised by Harris has been tried extensively in Zululand and in parts of the Congo. Other traps have been used in Tanganyika. The traps are advisedly placed in suitable sites near game animals in small preserves. In Tanganyika especially moving screens carried by fly boys have been devised for catching the flies by hand. These are carried through the areas where the flies are concentrated and many more flies are caught in this way by fly boys with nets and screens than in traps. However, it has been shown that even when trapping is intensively concentrated it cannot effect satisfactory extermination in the case of *G. pallidipes* and *G. palpalis* and it is even less successful with *G. morsitans*. Most of the traps examined by the writer in the Congo contained only a few flies though in some regions 100 to 200 flies are caught daily in such traps.

**Biological Control**—Humidity and temperature are important factors in the reproduction of *Glossina*. Temperatures between 25–30°C are very satisfactory for *G. morsitans* but above 35°C it very frequently dies. Burton and Lewis (1934) found that in temperatures above 40°C *G. morsitans* and *G. tachinoides* which may survive for short periods are more apt to do so in dry air than in moist. However the effects of humidity while very important to *Glossina* are also very complex. With a temperature of 30°C a relative humidity of about 44 per cent appears to be near

of the fly is attempted but the character of the vegetation so altered that the fly no longer inhabits such regions

However Swynnerton has emphasized that some four and a half million square miles is infested by one or more of the 21 species of *Glossina* and also by trypanosomiasis of domestic animals or of man. Hence it is evident that successful destruction of the fly can only be brought about in limited areas

On the Island of Principe the Portuguese have reported excellent results where the annual mortality from the disease formerly amounted to 83 per thousand of the population and the cacao industry was threatened with extinction through lack of labor. Here jungle clearing, drainage, diagnosis and segregation of the infection, destruction of possible animal reservoirs and finally actual destruction of the tsetse flies were employed. For the actual destruction of the flies natives were dressed in white and carried on their backs a dark cloth covered with bird lime. They were sent daily into the jungle and every night the flies caught were removed and counted. In 3 years it is reported 470,000 *Glossina* were destroyed. As a result of these sanitary measures sleeping sickness was said to be exterminated. Only the complete isolation and limited life of the island made such a result possible.

#### TREATMENT

The earlier the treatment is begun after infection the greater are the chances of cure. Many of the patients are likely to be suffering from ankylostomiasis or schistosomiasis and when these diseases are coexistent it is advisable to give preliminary treatment for them. Damage to the liver cells may be caused by such infections and may render the individual more susceptible to the toxic effects of the arsenical drugs used in the treatment of the sleeping sickness.

At the present time the 2 drugs most efficient in treatment are trypanamide, a synthetic arsenical preparation and Bayer 205.

Bayer 205 or *germanin* (the symmetrical urea of sodium m-amino benzol m-amino p-methylbenzol 1-naphthylamino 4,6,8-trisulfonate). The French have synthesized an equivalent of Bayer 205 under the name of Fourneau 309 or *moranyl*. The English equivalent is named *antrypol*.\* Bayer 205 is a white powder easily soluble in water. It is generally given intravenously but sometimes intramuscularly. The intrathecal injection of it cannot be recommended as pain, vomiting, headache, convulsions and twitchings have been observed after small doses given by this method. Also advanced cases derive no benefit from this method of injection. In animals infected with trypanosomes Bayer 205 is remarkably atoxic and the tolerated doses have been estimated to be many times the doses required for successful treatment and the destruction of the trypanosomes. It has also been found to exert a definite prophylactic action against trypanosomes. The average intravenous dose recommended for man has been 1 gm dissolved in 10 cc of distilled water. The total amount generally necessary to effect a cure is about 10 gm though trypanosomes usually disappear and are not observed again after 0.5 gm have been injected. Sometimes however they reappear.

Or Sulim or Naphuride (Winthrop)

game have been destroyed. The entomologist believes that in some localities the result has been successful. However in a number of other areas where game destruction has not occurred there has also been a natural recession of the flies and the efficacy of game destruction is still a very controversial one.

In connection with the wholesale destruction of game in parts of Africa it should always be considered that if the game is so reduced that the tsetse flies of the *Glossina morsitans* group are driven to attack man for food a much wider dissemination of these flies is likely to occur and hence further spread of human trypanosomes may result. Several human outbreaks attributed to this influence have recently been recorded. In parts of Tanganyika a much wider dissemination of *Glossina* has recently occurred, and since the institution of the Masai Reserve in Kenya Colony the fly belts with its confines have extended their boundaries and increased in number. In the case of *Glossina swynnertoni* (a vector of human as well as of animal trypanosomiasis), Lewis (1934) has obtained evidence which shows that there has been an actual invasion of it from Tanganyika Territory. Swynnerton has shown that *G. swynnertoni* in the presence of cattle may not attack man but where game is scarce it attacks man readily. More recently, Lewis has found that in the presence of an abundance of game and in the presence of cattle this fly very readily approached man and was also attracted to moving vehicles.

Concentration of the game animals in fenced preserves has also been used as a measure to attract the flies so that an intensive attack may be made upon them and their breeding grounds. Epidemics of sleeping sickness in man have occurred in which game animals have played no part whatever in the spread of the disease, the trypanosome being carried directly from man to man by the bite of the fly, and probably frequently mechanically. Lester (1939) emphasizes that in most places where one finds any concentration of population game is very scanty and that shooting anything near sleeping sickness settlements is most difficult because there is no game it has all gone. This has also been the writer's experience.

Swynnerton places little confidence in game extermination in fly control as he believes it is impracticable because of re-invasion difficulties in accomplishment and unreliability in a partially settled country. He especially recommends the annual burning of the bush by the cattle grazing natives, the building up of fly barriers of native bush preferably evergreen which are traversed slowly if at all by the fly, clearing of infested fly territory by native settlement, control of plant associations and methodical trapping of flies in certain territories until they are so reduced in numbers that human occupation can continue. He believes that roads through fly country can be made safe by clearing and that fly concentrations can be isolated in like manner. He emphasizes that extermination of the fly is out of the question.

Vegetational control of the disease has been emphasized as of greater value in which not only elimination of the favorable breeding places

were present in the blood or in the glands observed that all of the advanced cases which he treated with this drug relapsed. A significant fact in its use has been always the failure of the drug to reduce the cell count in the cerebrospinal fluid. Corson and McLean, Saunders and Chestermann have also observed similar results with Bayer 205. Saunders (1942) reports that of 36 cases followed up for 13 years or more, 34 of the 35 who had definite nervous involvement at the time of treatment have died of sleeping sickness.

*Tryparsamide* (the sodium salt of N-phenylglycineamide p-arsonic acid) is a much more valuable preparation in the treatment of advanced cases. The French equivalent is Fournieu 270 (*Orsanine*). It is a colorless crystalline powder freely soluble in water forming a neutral solution to litmus and is a potent trypanocide. It may be given by either the intramuscular or intravenous route, preferably the latter. The chemo-therapeutic index (difference between the curative and the maximum tolerated dose) is 1 to 2. On the other hand, in the case of atoxyl it is 1 to 1.

This drug was first introduced by Louise Pearce in the treatment of trypanosomiasis in the lower Congo in 1920 and its use continued there especially by Van den Branden. Kellersberger in Katanga has treated more than 8000 cases with this drug and has found it the most useful of all drugs in the general treatment of trypanosomiasis. Chestermann has also had a wide experience and has found the drug to be most effective by the intravenous route. The great danger in the use of the drug is that it may give rise to ocular symptoms and even blindness. Individual doses employed have varied from 1 to 4 gm. Kellersberger has finally placed the average dose at 0.045 gm per kg body weight. Even with 0.05 gm per kg ocular symptoms sometimes develop. Hence Chestermann recommends that in early cases in adults a start should be made with 0.04 gm per kg body weight. Children up to 12 years of age seem to tolerate the drug well and may be given double this dose up to 0.08 gm per kg of body weight. Kellersberger emphasizes that in order to be effective the drug must be pushed to the limit of safety. He formerly gave a series of 8 or 9 injections. His later procedure consists of a series of 15 weekly injections, the dosage being 0.045 gm per kg body weight. Chestermann has recommended a total of 12 weekly injections in all cases in which the central nervous system is involved and a second course of treatment should be given after from 1 to 3 months according to the condition of the patient. In early cases an apparent cure usually occurs.

*Tryparsamide acts less effectively in some instances if there has been prior administration of atoxyl or other arsenicals.* Ehrlich and Yocke have shown that the pathogenic trypanosomes readily become drug fast. For this reason Chestermann and Manson-Bahr suggest preliminary treatment with Bayer 205 followed by 6-8 weekly injections of tryparsamide. Between the two courses of treatment there should be an interval of 10-14 days until the urine becomes free from albumin before administering tryparsamide. Such combined treatment has also been recommended by McLean in Tanganyika, Duke in Uganda and Dye in Nyassaland.



in the peripheral blood after this amount. Generally the parasites are no longer visible in the blood stream some twelve hours after the injection of a number of trypanocidal drugs, but it is necessary that the dose of such drugs should be repeated at weekly intervals, or the parasites reappear. In initial infections a dose of 1 gm of Bayer 205 on the 1st, 3rd, 10th and 13th days is often employed. In exceptional instances with severe symptoms individual doses of 1.5 to 2 gm can be given to an adult man.

Edge (1938) reports that the standard treatment in Nigeria has been three 1 gm doses of antypol or Bayer 205 followed by five 2 gm doses of tryparsamide. However at the time the diagnosis was made all the patients were first given a trial dose of 0.3 gm of Bayer 205 in an attempt to detect occasional cases of idiosyncrasy to the drug. Before the trial dose method was instituted 7 cases of collapse with 1 death occurred among 6491 cases treated. In another area 2 cases were reported among 7942 patients following the trial injection of 0.3 gm of Bayer 205 while in two other cases the collapse was so severe that the patient might well have died if he had received the full 1 gm dose instead of the trial dose. Since the institution of the trial dose the number of collapses has been small and there have been no fatalities.

Kellersberger (1933) has employed the drug in some 4000 injections. He has found that on the average 0.102 gm per kg body weight is usually well borne. Bayer 205, however, has a cumulative action and is retained in the tissues for a considerable period so that the blood serum, cerebro spinal fluid and urine of the patient may contain and exhibit trypanocidal action when reinjected into trypanosome infected mice.

Dangerfield (1938) has found that small amounts of Bayer 205 were found in the plasma of animals several months after a course of injections which may explain its prophylactic action.

There are some disadvantages to its use. Thus it may cause urticarial and herpetiform toxic skin eruptions, conjunctivitis and stomatitis have also been noted. The drug is also irritating to the kidneys and in some cases after 3 or 4 injections the urine contains albumin and small yellow granular casts. Stitt has pointed out that it may cause nephritis with fatal uremia and when used subdurally is quite toxic for the nerve centers. Kellersberger (1933) has found that while the drug may cause nephritis this is temporary in most cases.

Attempts have been made to treat trypanosomiasis by giving this drug by the mouth while in some instances the trypanosomes disappeared from the blood, in the great majority of cases a relapse occurred in about a week.

Especially favorable results in the treatment of trypanosomiasis by injections of Bayer 205 have been reported by Kleine and Fisher. However, it has been shown that the treatment with this drug is usually only satisfactory in the early stages of the disease. After the central nervous system has become invaded it is ineffective. Kellersberger who found it to be of especial value during the febrile stages when the trypanosomes

Branden have reported on the trial of etharsanol. This drug was found to have an action comparable to tryparsamide but both of these drugs appear to produce optical disturbances more readily than tryparsamide.

Murgatroyd and MacQueen (1938) have recently studied the effects of treatment with neocryl (sodium succinanilo methyl amide para arsonate  $\text{CH}_3\text{NHCO}(\text{CH})_2\text{CO NH}-\text{C}_6\text{H}_4\text{AsO}(\text{OH})\text{ONa}$ ). Murgatroyd has treated 122 cases with this drug. Forty four of them had normal cerebrospinal fluids and 78 had pathological fluids. As a routine 10 doses of 0.045 gm per kgm body weight were given at weekly intervals. The drug produced a definite clinical improvement in practically every case treated although some were in a very advanced stage of the disease. In the early cases 30 finished the course of treatment. All were clinically improved. A few however experienced visual disturbances from which complete recovery was made and one case relapsed 16 weeks after finishing treatment. In the cases with pathological cerebrospinal fluid 46 finished the course of treatment and of these 35 were clinically improved. However in 4 cases trypanosomes failed to disappear from the spinal fluid. Three patients suffered from toxic effects of the drug the most serious of which were the disturbances of vision. Murgatroyd believes the results resemble those which are obtained in treatment with tryparsamide. MacQueen who treated 60 cases with neocryl noted rapid clinical improvement and both the blood and gland juice were cleared of trypanosomes at the time of the second routine examination after treatment. Disturbances of vision were noticed in 2 cases one of which became completely blind and died within a month of cerebral trypanosomiasis.

Acres (1940) has also employed neocryl and found that it usually produces rapid clinical improvement and in first stage cases is of value. However its effect may be only temporary and cases treated in the second stage of the disease frequently relapse. Twenty one cases were treated and observed over a period of at least 2 years. Of 12 patients treated in the second stage only 3 were cured. Apparently tryparsamide is still the most valuable drug for the treatment of trypanosomiasis.

Atoxyl (or soamin) was formerly used but it is much more apt to cause optic neuritis, atrophy and blindness. It also causes gastro intestinal irritation.

Tartar emetic (sodium or potassium antimony tartrate) has also been employed for treatment. It is used in a 1 per cent solution in physiological salt solution sterilized at  $100^\circ\text{C}$  for 15 minutes. It may be given in a dosage of 5 cc. to adults administered slowly by the intravenous route. If a cough supervenes showing intolerance of the preparation its administration should be immediately stopped. Others advise that one should commence treatment with  $\frac{1}{2}$  gr dissolved in 10 cc of sterilized distilled water. This intravenous dosage should be increased by  $\frac{1}{2}$  gr on each occasion until a limit of 2 gr is reached. Some have recommended injections of tartar emetic on alternate days others twice weekly and some once a week. Tartar emetic was formerly given

**Optic Neuritis**—Many patients are very sensitive to arsenical preparations such as tryparsamide. Opinions differ as to the amount of tryparsamide which may provoke optic neuritis. Manson Bahr reports two cases where blindness ensued after 13 gm had been injected. Lauterborg observed blindness in 7.4 per cent of his patients who had received weekly doses of 2 or 3 gm of tryparsamide. Kellersberger reports that the blindness rarely came on suddenly. However there is a danger of injury to the optic nerve and cases of blindness occurred even when patients were carefully watched. Manson Bahr found that objective signs of eye damage are not manifest early enough to enable one to prevent complete blindness from developing. The fundus remains normal for a long time and the pallor of the disk sets in quite late. The premonitory symptoms may be photophobia, lacrimation, pain of the eyes, and dimness of vision. Jamot found that of 25,638 patients treated with tryparsamide, 233 developed ocular trouble and in 30 there was amblyopia and in 17 amaurosis. The action of arsenic is often a delayed one and symptoms of neuritis may develop even after cessation of treatment.

Juler (1940) has noted that some 20 cases of dermatitis sometimes resembling exfoliative dermatitis have followed the administration of tryparsamide. It has been suggested that this increased toxicity may be due to some variation in manufacture of the preparation.

The statement occurs in a number of text books that tryparsamide while very effective in the treatment of infections with *T. gambiense* is of little value in the treatment of *T. rhodesiense* infections. In view of the fact that these trypanosomes are apparently identical this statement requires modification.

Hawking (1940) has demonstrated that the degree of trypanocidal activity produced by tryparsamide in the cerebro spinal fluid of patients treated with this drug is insufficient to exert much effect upon freshly isolated strains of *T. rhodesiense*. Nevertheless the activity was greater than in patients treated with neocryl or undecane diamidine. In 1941 it was found that the trypanocidal activity produced by tryparsamide in the blood is somewhat greater than in the cerebro spinal fluid but that the arsenic disappears from the blood very rapidly after injection.

If the degeneration of the central nervous system has not extensively progressed and when the cerebro spinal fluid does not contain large numbers of cells the drug generally gives gratifying results. After cerebro spinal symptoms have developed while the drug is not as effective from 17 to approximately 50 per cent of cures have been reported by different observers. A normal cell count and albumin content of the cerebro spinal fluid as well as the physical improvement in the patient's mental capacities gives evidence of cure after the cessation of treatment.

**Treatment with Other Drugs**—A number of other arsenical compounds have been recommended for treatment. These drugs include etharsanol (monosodium salt of 2 p arsono arsilino ethanol) and proparsanol (monosodium salt of 3 p arsono anilino propanol) each of which contains 20 per cent of arsenic. Stratman, Thomas, Lovenhart and Van den

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intramuscularly, but it produced great pain. The use of the organic compounds of antimony is fully discussed in Chapter V.

Certain strains of trypanosomes have also been found to become antimony fast as well as arsenic fast. Hence some authorities as Rod hain and Van den Branden, have used tartar emetic in connection with other drugs notably atoxyl.

*Stilbamidine and Pentamidine*—British investigators have recently studied the effects of 4,4'-Diamidino Stilbene, (Stilbamidine) in human and animal trypanosomiasis. Mc Letchie (1941) in a small series of cases in Nigeria without marked involvement of the nervous system found the former to be apparently as effective as Bayer 205. Patients with mild infections received an average total dose of 8.8 mg per kilo. However Harding (1941) found improvement with this drug only in cases in which the cerebrospinal fluid was not abnormal. In the Gambia, Bowesman (1941) has obtained good results with 4,4'-Diamidino Stilbene given intravenously in doses of 1.0 mg per kilo of body weight twice each week to a total of nine to ten injections. He considers larger doses are toxic and unsafe. A still more recent preparation is 4,4'-diamino diphenoterpene (Pentamidine) or M & B 800. Saunders (1941) has reported upon 14 cases successfully treated. In all cases the peripheral blood was sterilized by four injections and most cases were cured after twenty injections. Lawson (1942) has treated fifty three cases with Pentamidine in Uganda. Patients eleven years and over were given 0.1 gm at each injection intravenously, ten injections being generally given and the course of treatment completed in ten days. On examination two to three months later, 41 were clinically cured, 3 much improved, 4 improved and 4 unaltered or worse, 1 had died. Lawson considers this is probably the best drug that has been produced for the early treatment of cases of sleeping sickness. He however cautions that no case with a cerebrospinal fluid cell count of 30% or more should be given Pentamidine unless the case can be carefully followed up. Daubney and Hudson in the treatment of cattle infected with *T. congolense* with these drugs Stilbamidine and Pentamidine, found that even in poisonous doses they did not produce a complete cure even though some of the animals succumbed to the effect of the drug. Yorke has emphasized that these drugs, while efficient, are poisonous and must be used cautiously in the treatment of human cases.

Van Hoof et al (1944) believe that Pentamidine cures easily and safely early cases of gambiense sleeping sickness and may replace Bayer 205 in arsenic fast cases but they think it is as a preventive that it seems to have the greatest value as it is eliminated slowly and accumulates in the body retaining a strong trypanocidal action which prevents an infection by flies as well as by mechanical transmission. Volunteers infected with a single dose of 0.001 or 0.003 gm per kg resisted for 10 to 12 months repeated bites of infective tsetse flies.

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## Chapter IV

# SOUTH AMERICAN TRYPANOSOMIASIS

### DEFINITION AND SYNONYMS

**Synonyms**—Brazilian trypanosomiasis Schizotrypanosomiasis Chagas disease Opilação Enfermedad de Chagas

**Definition**—An infectious disease occurring in certain provinces in Brazil and occasionally in other areas in South and Central America caused by *Trypanosoma cru* 1 (*Schizotrypanum cru* 1) (Chagas 1909) and commonly disseminated by reduviid bugs especially *Triatoma megista* and *T. infestans*. Pathological changes are produced by the destruction of the endothelial and tissue cells of the body by the development of the parasite within these cells. The disease especially affects children and acute and chronic forms have been described. In the acute stage febrile disturbances associated with facial oedema adenitis and anaemia are common symptoms. In the chronic stage the symptoms depend especially upon the localization of the trypanosomes in the different organs and tissues of the body especially the heart central nervous system thyroid or suprarenal glands.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History**—The Brazilian scientist Carlos Chagas in 1909 discovered in the intestine of a reduviid bug *T. megista* an organism resembling a trypanosome. The infected bugs were allowed to bite a monkey and the trypanosome was afterwards found in the blood of this animal. Subsequently he found the same trypanosome in the blood of a child suffering from fever anaemia and swelling of the lymphatic glands. Thus the parasite causing the disease and the insect transmitting it were discovered before a human case of infection had been detected. Chagas later was able to show that other animals than man harbored the parasite and wrote of the various symptomatology of the human infection. In 1916 he published an account of 29 acute cases which he had encountered in the intervening years. All were in infants and young children. In all parasites were found in the blood and of 23 cases which he was able to follow for some time 11 died. Villela and Vichalho (1923) also working in the State of Minas Geraes in Brazil by the inoculation of the blood of 19 supposed chronic cases of the disease into guinea pigs found the trypanosome in the blood of 5 of the guinea pigs. In 1919 cases of infection were found by Tejera in Venezuela and by Escomel in Peru.

Chagas originally believed that during a certain stage of the life cycle of the parasite in man *Trypanosoma cru* 1 multiplied by schizogony and hence he established



found (60%) In Panama up to 1937, 19 positive cases had been diagnosed by Miller (1931) Clarke and Dunn and others. However, Clarke (1939) reports that through the use of the complement fixation test by Johnson and Keiser and Clarke the total number of positive cases diagnosed by this test has been increased to 62. The trypanosome has been reported in human beings in Ecuador and Paraguay but not in southern California or Arizona though *Triatoma* infected with this parasite have been found in these countries. However Mazzotti and Brumpt in 1939 encountered the first 2 cases in Mexico.

Gasic in the latter half of 1937, spent some time in the Province of Santiago Chile examining the blood of residents for infection and making animal inoculations of the blood but was unable to discover any human



FIG. 46.—Sch of *Trypano. cruzi*. (After Muhl.)

case until 1938 although the *Triatoma* were earlier found infected. In 1916 Kofoid and McCullough found *Triatoma* infected with this parasite in California and in 1938 Kofoid reported that the parasite could be experimentally transmitted to a wide range of mammals in California through infection from this bug. It has been known for many years that the bite of the so-called kissing bug, or the cone nosed bug of California (*Triatoma protracta*) is exceedingly irritating and in some instances the person bitten has been reported as ill for weeks afterwards. However no case of human infection with *T. cruzi* has been reported outside of Central or South America. Packchianian (1939) has found natural infection of *T. gerstaeckeri* in Texas. Of 100 bugs examined 92 were naturally infected and the infection transmitted to animals and experimentally in one case to man (1941) by *T. heidemannii*.

#### ETIOLOGY AND EPIDEMIOLOGY

**Etology**—During the febrile attacks *T. cruzi* can often be found in the circulating blood though usually only in small numbers. The trypanosome is pleomorphic in character having 2 phases in its life cycle, one in man and other mammals and one in the transmitting insects. In the



for it the genus *Schizotrypanum* naming the parasite *Schizotrypanum cruzi*. Subsequently however it was shown that multiplication in the mammalian host (though occurring within the cells in the leishmania stage) is not by schizogony but by the usual method of binary fission hence it was thought appropriate to retain the trypanosome in the genus *Trypanosoma* and to employ the original name given it by Chagas *Trypanosoma cruzi*.

Hoare (1936) takes this view. However Dias (1939) who has studied the question in detail believes that the genus *Schizotrypanum* should be retained. He points out that *Schizotrypanum* has peculiar morphological characters which assimilate it to *Leishmania* in the intracellular period and to *Trypanosoma* in the blood stage. Flagellates belonging to this genus are characterized not only by the morphology of the trypanosome form but also by the evolution in the vertebrate's organism. It differs by its evolution in the insect and its mechanism of transmission which are common to the non pathogenic trypanosomes. He believes that *S. cruzi* cannot be rigorously included in either the Genus *Trypanosoma* or *Leishmania* as it is easily distinguished both by its morphology and by its biology.

**Geographical Distribution**—In Brazil, the disease has been reported in the states of Minas Geraes, Sao Paulo and Goyaz. It has also been observed in Argentina, Uruguay, Venezuela, Peru, Bolivia, Chile, Costa Rica, San Salvador, Panama, Guatemala and Mexico\*. The different reduviid vectors have a much wider distribution extending in the western hemisphere from the Argentine Republic in South America to California and Utah in North America. In view of these facts, Brumpt, and more recently, Kofoid have suggested the possibility of finding that the distribution of the human disease may be over a much wider area than at present known. However, it seems likely that climatic and other ecological conditions especially social influences operate to prevent the coincidence of the geographical distribution of the disease in man with that of its several vectors.

**Prevalence**—Although Chagas's disease has been known since 1909 the number of human beings who have subsequently been found to be actually infected with the trypanosome has been until recently comparatively small. Up to 1937, the only other Brazilian state besides Minas Geraes in which definite instances had been found was Sao Paulo in which 4 cases were reported. Talice (1939) reported the first case in the state of Rio Grande del Sur. Outside of Brazil the largest number of cases recorded has been in Argentina. Mazza (1937) reports that between 1932 and 1936 the number of acute cases reported in Argentina amounted to 109. However a number of these had not been published. In 1939 he reported that the total number up to the end of June 1938 had reached 370. Of this total 345 had been diagnosed by direct examination of the blood. By April 1940 more than 500 cases had been observed and by Dec. 1941 630 cases. Two definite cases have been recorded from Peru. Subsequently Citola (1937) wrote that he had seen a number of additional cases of the disease in Peru but the description of the trypanosomes found rendered the diagnosis doubtful. Until 1937, no cases had been reported in Uruguay. However, Talice discovered 11 cases in that country in 1938 and by 1941, 49 cases had been found. Five cases have been reported from Venezuela, 3 by Tejera. Iriarte (1937) believes the disease occurs more frequently there on account of the high rate of infection of *Triatoma* he

\* Mazza (1943) reports that with the discovery of the infection in man and animals in Bolivia all countries in South America are now infected.

introduced into the conjunctiva or mouth in other instances producing infection

**Cultivation**—Cultures of *T. cruzi* can be obtained in blood broth or on NNN medium. The organism is said to lose its virulence on prolonged cultivation. The young cultural forms have been found infective for experimental animals. The forms found in culture are similar to those seen in the intestinal tract of the transmitting insect.

Lwoff (1938) has found that abundant growth of *T. cruzi* will take place in serum to which both haematin and ascorbic acid are added. However the suppression of either or both of these substances interferes seriously with the cultivation and they appear to be essential factors for its growth. The serum also contains an essential factor for the cultivation for its removal is likewise injurious. What the necessary substance in the serum is has not been discovered but it was thought that it possibly was cholesterol. Romana and Meyer (1942) in the study of chick-embryo culture tissues employed Carrel's technique with light modifications and have been able to follow the complete cycle of the parasite from the flagellate stage to that of the trypomastote.

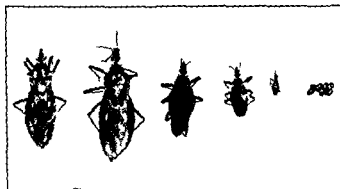


FIG. 48.—Life cycle of *T. megista*. In its carrier (the *Scutiger mormon*).

**Transmission**—The common method of transmission of the disease is through the reduviid bugs especially *Triatoma megista*. This species was formerly described in the literature under the name of *Conorhinus megistus* or *Linus megistus*. More recently Pinto (1931) has reclassified the species under the name *Panstrongylus megistus*. In Northern Argentina and Uruguay the more common transmitting species appears to be the uruchaca (*Triatoma u. festans*). However the parasite is capable of developing in a number of other species of *Triatoma* and allied genera as *Rhodnius Eratyrus* and *Eutriatoma*. Some 20 species of Triatomidae have so far been found naturally infected with *T. cruzi* and capable of transmitting infection (Lent and Pifano 1939). In California *Triatoma protracta* has been found infected by Kofoid while Reichenow (1934) in Guatemala, found the transmitting bug to be *T. dimidiata* and Clarke and Dunn (1932) in Panama have shown that *Panstrongylus geniculatus* is the usual transmitting arthropod but *Eratyrus cuspidatus* was also found naturally infected.

blood the parasite appears as a typical trypanosome and in the endothelial and tissue cells it appears as the leishmania form and subsequent transformation stages. In the transmitting insects the developmental stages including crithidia and metacyclic, trypanosomes occur in the mid and hind gut respectively of the infected bugs. The trypanosomes as seen in the blood are frequently spindle shaped and about  $20\mu$  in length. Both long slender and short broad forms occur in the blood. However this variation in morphology has no sexual significance as was formerly believed. The nucleus in both forms is central in position. The kinetoplast is oval in shape and on account of its enormous size is characteristic. It is situated near the posterior end. In well stained preparations the



FIG. 47 — *Trypanosoma cruzi* in blood of child with acute type of Brazilian trypanosomiasis (Mac from Doflein after Chagas)

kinetoplast is seen to consist of a dot like blepharoplast and a larger oval parabasal body. The root of the flagellum or acroneme arises from the blepharoplast and extends along the edge of a narrow, undulating membrane with few folds and projects at the anterior end of the body as a free flagellum. In blood films which have been fixed and stained the trypanosome is usually curved forming C or U shaped forms. It should be noted

that dividing forms do not occur in the blood which is a point of differentiation from other human trypanosomes.

Later the trypanosome invades the tissue cells and loses its undulating membrane and flagellum. The organism then divides by binary fission. By repeated further divisions the leishmania forms eventually fill and destroy the infected cells. In films made from the organs stained with Giemsa's stain (especially of the tissues of the heart and voluntary muscles and sometimes of the thyroid gland and brain) these leishmanni forms are seen to occur in intracellular cysts the cyst usually being filled by them. They are round or oval in shape measuring from 3 to  $5\mu$  in diameter and contain a nucleus and kinetoplast. The invaded cells are later destroyed and the parasites liberated as leishmania, leptomonas, crithidia or trypanosome forms. Only the trypanosome forms are found in the peripheral blood. Some of these trypanosome or leishmania forms enter new cells and the developmental cycle is repeated.

Infection of the bug occurs when it bites an infected individual or other mammal. Trypanosomes multiply in its gut by longitudinal fission and first undergo development into non infective crithidia in which the kinetoplast is at the anterior end and the nucleus central. Intermediate forms with the kinetoplast in variable position and metacyclic trypanosomes with the kinetoplast at the posterior end and a well developed undulating membrane and flagellum subsequently appear. These metacyclic trypanosomes are infective and are passed out of the hind gut in the insect with the faeces. They may contaminate the wound made by the insect when biting man or enter through abrasions of the skin or they may be

have a length of 0.45 m. The head is small and pointed and the ears large. The fore legs have usually 5 toes each ending in very sharp and strong claws. The middle claw is greatly increased in length. By means of these claws it can excavate the earth with great rapidity. *Tatusia notemneis* has only 4 digits on its anterior paws. These animals live in underground burrows and come out at night to feed on carrion, worms, insects or fallen fruit. If touched they will either shrink up into their armor and feign death or run away quickly. The tatus while usually classified with the edentates are not completely without teeth since they possess molars. These animals are not known to be of any value though the flesh of several species is considered by the natives as good to eat. The flesh of other species has a pungent disagreeable odor.

In Panama Clark and Dunn discovered a number of species of bats naturally infected as well as the armadillo *D. novemcinctus fenestratus* and the opossum *Didelphis marsupialis extensus*. Chagas, Mazza and others have also found the opossum infected in South America. In California Kofoid found the species *Didelphis virginiana* and the wood rat *Neotoma fuscipes* to be naturally infected and that *T. protracta* was apparently the transmitting agent.

*Triatoma uhleri* was also discovered to be naturally infected with *T. cruzi* in Arizona and in Texas. Packham (1940) found both *T. geckleri* & *T. heidemanni* naturally infected. Wood (1938) however in a relatively wide survey in Arizona, New Mexico, Texas and Utah of the blood of wood rats and faeces of *Triatomidae* found no evidence of infection with *Trypanosoma cruzi* in nature though the bugs were subsequently infected experimentally in the laboratory. Chagas as early as 1909 found the monkey *Chrysomys xanthureus* naturally infected in Brazil and regarded it as a host for human infection while Malamos (1935) found *T. cruzi* in 4 monkeys *Macacus cynomolgus* that were imported into Germany and examined at Hamburg. The monkeys had arrived from Java. If they were naturally infected this would be the first record that *T. cruzi* had been encountered outside of South America. *Triatoma rubrofasciata* is found in Java and has been found to harbor trypanosomes but these have so far been identified as *Critidia corrhini*.

The method of transmission of *T. cruzi* has been considerably disputed. Two hypotheses have been advanced. Chagas originally believed that transmission takes place as the result of the bite of the insect. Many other observers including Brumpt believe that infection occurs through the contaminative method, the faeces of the arthropod being rubbed into the mucous membrane or the wound caused by the bite of the insect as in scratching. Owing to various discrepancies in the literature about the exact method of infection, Cardoso (1938) has reinvestigated the subject using in his experiments infected *T. infestans* and mice. Freshly passed infected faeces of the *Triatoma* were first placed on the intact mucous membrane (ocular 4 cases, vaginal 1 case, buccal 2 cases and rectal 2 cases). In all but 2 instances 1 vaginal and 1 rectal infections resulted. In the second series of experiments infected dejecta were placed on the intact abdominal skin of a mouse. All of 10 such experiments proved negative. In a third series infected dejecta were placed on the lightly scarified skin. All of the 10 experiments proved positive. In a fourth series of experiments an attempt was made to ascertain whether *T. infestans* actually transmitted the infection by means of its bite. Chagas in earlier years believed that the trypanosomes were present in the salivary glands of the

Four species of bedbugs including *Cimex rotundatus* and *C. lectularius* have also been experimentally infected. The ticks *Amblyomma cajenense*, *Ornithodoros moubata* and *Rhipicephalus sanguineus* have been experimentally infected and shown to be capable of transmitting infection.

Under ordinary conditions the *Triatoma* becomes infected in from 8 to 10 days after biting the infected human being or animal and it may remain infected for as long as 2 years. In the tick *Ornithodoros moubata* Mayer found infected trypanosomes in the intestine as long as 5 years after the infected feed which were still highly virulent for mice. He also showed that infection may sometimes be conveyed hereditarily in *T. megista*.

**The Family Triatomidae**—The *Triatoma* have popularly been known in Brazil as barbeiro and in the United States as the kissing assassin or cone nose bug. The family includes 9 recognized genera of which the following are medically important: *Triatoma*, *Rhodnius*, *Panstrongylus*, *Eratyrus*, *Eutriatoma* and *Psammolestes*. These genera have been differentiated by Pinto with respect to the place of insertion of the antennae in relation to the eyes, the length of the proboscis, and the relative length of the joints of the proboscis. They are large blackish insects with numerous symmetrically arranged red markings and have been found in the Western Hemisphere between 41° northern latitude Utah and 41° south Bahia Blanca. Some species have been described from Asia, South China, Sumatra and Madagascar. In all more than 40 species are known. The species which have been found to be especially concerned in the transmission of the human infection in different localities have already been named above. The *Triatoma* are mostly dependent on wild animals for feeding, but certain species have become adapted to human habitations. They are avid blood suckers though they puncture the victim's skin and withdraw the blood with little or no pain. The lesions are commonly produced on the exposed parts of the skin and particularly on the face and eyes and lips, hence the name kissing bug.

The infection is believed to be commonly transmitted by the adult forms of the *Triatoma*. However, the bites of the larvae or of the nymphs have been shown to be infective experimentally for animals. Mazza recently examined 1712 specimens of *T. infestans* adults, nymphs and larvae captured in the sleeping rooms of dwellings in Brazil. A total of 576 or 33% were found infected, 300 or 36% of the adults were infected, 220 or 24% of the nymphs and 56 or 22% of the larvae.

**Animal Hosts**—Under experimental conditions practically all laboratory animals can be readily infected with *Trypanosoma cruzi* and dogs and especially cats have been found naturally infected and may serve as reservoirs of the parasite. Chagas first showed many years ago that cats were naturally infected in Minas Geraes, and Talice (1938) has found them naturally infected in Uruguay. In South America armadillos are regarded as especially important reservoirs for *T. cruzi*.

Chagas demonstrated in Brazil that the tatu or armadillo (*Tatusia* or *Dasyurus novemcincta*) may serve as the natural reservoir for *Trypanosoma cruzi*, from which animal the parasite was transmitted to man by *Triatoma megista* or *T. geniculata*. Subsequently some 5 species were shown to be naturally infected with this parasite: *Dasyurus novemcinctus*, *D. sexcinctus*, *D. unicinctus*, *Cabassus unicinctus* and *ChaetophRACTUS vellerosus*. Craig and Faust (1943) have listed other species.

The tatu or armadillos are animals whose body, legs and tail are covered with an armor of articulated scales which, however, do not prevent the animals from running quickly. The largest species attains a length of 0.86 m. without the tail which may

to the nymph although the nymph is capable of sucking the blood and becoming infected and has been found naturally infected

Mayer obtained a positive result in the hereditary transmission of this trypanosome in *Triatoma megista* which he believes may explain when coprophagy and cannibalism are excluded continual infection of these insects in regions where human cases of the disease do not exist He separated the eggs from the adult insects before they were hatched and the larvae which were hatched from these eggs were fed on healthy rats and mice Among many failures with hundreds of larvae thus fed he obtained in one experiment with 60 to 80 larvae a positive result He then examined the increment of 58 larvae of this lot and found 18 positive for the parasite These larvae remained continually infected He points out that while under the artificial condition of the experiments hereditary infection may thus occur it does so only rarely When it does occur however a large percentage of the brood may be found infected

Kofoed emphasizes that for many years it has been known that the bite of the cone nosed bug *Triatoma protracta* of California and the arid south west is exceedingly irritating and in some instances the person bitten has been ill for weeks afterwards But no case of human trypanosomiasis comparable to that in Brazil has ever been reported north of Central America except the two cases found by Brumpt (1939) in Mexico that have been referred to

The *Reduviidae* may become adapted to living in the houses of the lower classes of the people They are also commonly found in the out houses such as pig sties stables and chicken houses The insects tend to remain in the same house where they have become infected but leave it is said if it is abandoned by man So far infection has been found only among the poorer classes of natives in the endemic regions Sex has no influence upon infection but age is an important epidemiological factor as the disease is most common in children from a few months to two years of age The bugs have frequently been observed biting children while they are asleep and without their awakening so that infection usually occurs without the knowledge of the individual The insects bite at night and hide in the cracks thatched walls and roofs of the houses during the daytime

**Prevalence**—Yorke (1937) comments on the fact that the number of human beings reported as actually infected with *Trypanosoma cruzi* is remarkably small in view of the ubiquity of infected bugs in many parts of South and Central America Excluding Minas Geraes he was only able to find in the literature reports of 117 cases in human beings This is the more surprising as the parasite had evidently been carefully sought for in many places where the disease was reported as endemic Clark and Dunn noted that the clinical and pathological records of the Panama Canal for 27 years showed no entry of a case of Chagas's disease Clark during his field surveys for malaria in Central America where over 65 000 men women and children were examined found no case of infection with *Trypanosoma cruzi* in Panama up to 1930 However Muller described

insect and were injected at the time of biting and Torres reported infection in kittens by the bites of *T. megistus*. However Dias (1932) found that *T. cruzi* may remain in the stomach of the bug for as long as 8 days after biting and that such apparent infections by biting were due to regurgitation of these trypanosomes into the puncture wound. Cardoso took especial care in his experiments to ascertain that the wound did not become infected with the faeces of the bug. In one of 10 biting experiments a positive result was obtained. A month later the experiment was repeated with the same infected bug and in this case all ten observations were negative. Cardoso thinks that in the single positive case regurgitation of the trypanosomes from the intestine may have occurred at the time of biting. Brumpt (1939) and also Denecke and von Haller (1939) have also found that the bite alone without regurgitation does not cause infection. Brumpt (1912) reported that *T. cruzi* would pass not only through the healthy conjunctiva but also through the normal skin of mice. Evans Chagas (1935) in experimenting with 3 human volunteers found that the trypanosome could not pass through the unbroken skin but that it could pass through the conjunctiva. A human case of infection was obtained by placing the faeces of the infected *Triatoma* directly into the eye. Twelve days later fever occurred and the patient's blood 14 days after the inoculation was shown to be infective to guinea pigs.

It has been suggested that transmission of *T. cruzi* may occur through the milk of an infected nursing mother or by coitus as in dourine. The latter method of infection has not been demonstrated in man. However Nattan-Larrier produced infection in mice by placing material containing the trypanosome in the healthy vagina of animals and also showed that *T. cruzi* passes into the milk of infected animals. Mazza and his associates (1936) report the case of a woman aged 30 with symptoms of Chagas's disease who was confined 2 months later. No parasites could be found in the child's blood and 10 days later they left the district. Eleven weeks later the mother and child were again examined. The blood of the child contained many trypanosomes and the lymphatic glands, liver and spleen became enlarged but no cutaneous lesions were present. Examination of the mother's milk on the same day showed 3 *T. cruzi* in the centrifuged deposit. They were also found in the milk 6 days later. However, no parasites were found in thick drops of the mother's blood.

**Epidemiology**—The *Reduviidae* are vicious biters and from their biting chiefly about the face have been called 'barbeiro' (or barber) by the natives of Brazil, and the 'kissing bug' in the United States. Both the male and female carry the infection. The adults can fly short distances in search of food and infected bugs have been found far from human habitation. Armadillos may serve as reservoir hosts and infected bugs have been found inhabiting the burrows of the armadillo near dwelling huts and it is thought that when the burrow is abandoned by the animal the bugs migrate to the nearest source for food which may be man. The larvae and nymphs which also feed on blood are wingless. It has generally been believed that the parasite is not transmitted hereditarily.

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a case there in 1930 and up to 1937, 19 cases were reported. Subsequently Kelser diagnosed other cases by means of the complement fixation test. The recent discovery of numerous cases in Argentina has been emphasized by Mazza (1940).

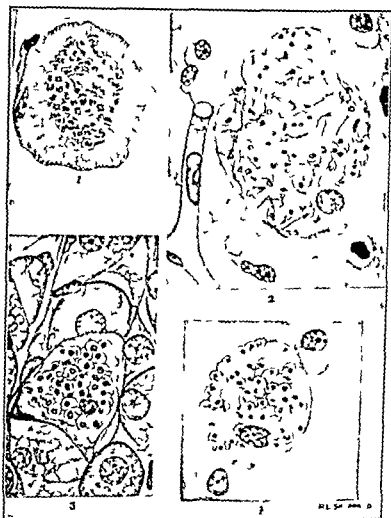


FIG. 40.—*Schizotrypanum cruzi* developing in the tissues of the guinea pig. 1 Cross-section of a striated muscle fibre containing *Schizotrypanum cruzi*. Note dividing forms. 2 Section of brain showing a *Schizotrypanum* cyst within a neuroglia cell containing chiefly flagellated forms. 3 Section through the suprarenal capsule fascicular zone. 4 Section of brain showing a neuroglia cell filled with round forms of *Schizotrypanum*. (From Low in Sleeping Sickness Bulletin after Vianna.)

It seems probable that *T. cruzi* is naturally a parasite of armadillos and opossums and is only occasionally and accidentally inoculated into

man Only in comparatively recent years has the disease been reported by laboratory workers outside of Brazil

### PATHOLOGY

The chief pathological changes produced by *Trypanosoma cruzi* are degeneration and destruction of the invaded cells of the body by the development of the parasite within them and eventually a fibrosis in the affected tissues In acute cases the parasites have been found in almost every organ in the body The most marked lesions have usually been reported in the heart and brain and liver The involuntary muscles and adrenals have also been frequently invaded by the parasites Continued division of the leishmania form of the parasite in the cells convert the lesion into a sort of cyst This process going on in different organs apparently accounts for the extreme variation in symptomatology At autopsy the heart is usually found enlarged Microscopically there may be evidence of diffuse myocarditis In sections studied by the writer many of the fibres were heavily parasitized In places there was a perivascular infiltration with endothelial cells lymphocytes and a few plasma cells Endothelial leucocytes were also seen scattered between the muscle fibres The muscle fibres themselves were widely separated from one another and in places there was some proliferation of the nuclei The fibres sometimes show fragmentation and hyalin degeneration The most conspicuous change was the presence of large nests of parasites between the muscle fibres Some of the fibres were bulged out as a result of being stuffed with the parasites The parasites lie either in rounded clumps or in longitudinal bands Profuse myocarditis with more extensive cellular infiltrations has also been reported The epicardium and endocardium sometimes show in places cellular infiltration and nests of parasites

Mazza and Romana (1935) described the pathological conditions found in the heart of a child who died of bronchial pneumonia 5 weeks after the onset of an acute attack of Chagas's disease The heart muscle fibres were normal but the interstitial tissue showed an intense infiltration by monocytes which separated the muscle fibres one from another There was also a hyperplasia of the connective tissue with beginning fibrosis No parasites were at first found Later in a few large cells they were discovered It is believed that if the heart lesions are not sufficiently severe to kill the child in the acute stage the infiltrated areas undergo fibrotic changes E v Chagas (1935) and Mazza (1939) have also found in other chronic cardiac cases hyperplasia of the connective tissue and fibrosis with parenchymatous infiltration

C Chagas first emphasized the pathological changes in the heart The predilection of the parasite for the heart muscle was more recently shown by E v Chagas in 1934 He inoculated with infected blood a volunteer suffering from an inoperable cancer This patient died 6 months later of carcinoma His blood showed trypanosomes and at the autopsy the only organ found infected was the heart leishmania forms being demonstrated in the muscle Johnson (1938) has recently studied the

pathological changes in the heart in dogs. In his opinion the lesions observed could be accounted for on the basis of mechanical action of the parasites. Focal lesions were most numerous in the layers adjacent to the epicardium and endocardium with auriculo ventricular conjunction.

The skeletal muscles are also a seat of election for the parasites and their multiplication. The pathological changes found in them are similar to those which occur in the heart.

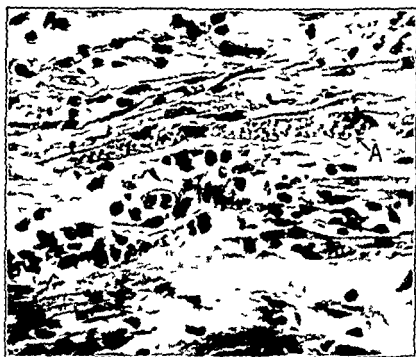


FIG. 30.—South American trypanosomiasis. Section of heart showing (A) invasion of muscle fiber by *Schizotrypanum cruzi*. (Section courtesy of C. Chagas—Army Medical Museum Photo No. 46926.)

In some cases pathological changes have been noted in the brain. The brain and meninges are sometimes congested and oedematous and scattered throughout the substance of the brain numerous small inflammatory foci are occasionally present. In addition to the pathological changes noted by Chagas, Vianna was the first to describe the foci of encephalitis and myelitis in human cases and to show that they are readily produced in animals. These lesions were also studied by Torres and Viella in greater detail who showed that the cells making up the foci of encephalitis and myelitis are neuroglia cells together with mononuclear cells. It was sometimes difficult to make out the nature of the parasitized cells owing to their distortion by the presence of the trypanosomes within them. Crowell, who studied in Brazil tissues from Chagas's cases, pointed out that parasitization of the nerve cells proper is practically never seen but

in the semilunar ganglia of a heavily infected puppy he found a parasitized cell that was unmistakably a ganglion cell. Mazza (1938) has observed a fatal case in an infant of 2 months who died on the 6th day of the disease. The most noteworthy point at autopsy was the meningio choroiditic lesions with encephalitis. He had observed this condition repeatedly in experimental animal infections but not in human cases.

Lunderberg (1938) studied at autopsy a fatal case due to *T. cruzi*. Focal encephalitis due to this parasite was found but in the mid brain only. There was also acute myocarditis and hypertrophy and dilatation of the heart. The parasites were found in the heart, brain and prostate.

Enlargement of the liver has frequently been observed. On section the organ may show cloudy swelling or extreme fatty degeneration. The parasites have only been reported in the liver in comparatively few cases but their presence has been observed in a few instances in the Kupffer cells. The spleen is usually somewhat enlarged and congested but in the great majority of fatal cases the lesions in the spleen have been complicated by malarial infection. Mazza and his associates have reported the presence of parasites in the spleen but frequently they have not been detected in the spleen. The lymphatic glands are often enlarged and on section may show congestion with some lymphoid hyperplasia. The parasites have been found at postmortem in a number of acute cases in the thyroid, suprarenals, ovaries and testicles. Mazza (1939) has observed nodular formations generally in the center of the nodules consisting of lymphocytes in a network of reticular histiocytes with parasites.

### SYMPTOMATOLOGY

The symptomatology is variable. The variation in the severity of the disease in different places is very striking. In many individuals in whose blood the trypanosome has been found no evidence of the disease or of recent illness has been discovered but in a majority of the cases there has been a mild febrile disturbance associated with more or less facial and other oedema and enlargement of some of the lymphatic glands. It is especially a disease of children in the endemic areas and it is especially in children that the symptoms observed have been more severe and as a rule the younger the child the more severe are the symptoms. However the disease also occurs in adults. In adults a number of infections have been discovered only as the result of systematic examination of the blood of a large number of individuals.

Some clinicians believe that there are no definite clinical characteristics. Miller found in his study of the Panama cases that the manifestations of disease were practically absent and the trypanosomes disappeared from the peripheral blood in the course of a few weeks without treatment with any specific drug.

E v Chagas (1934) in the study of a human case experimentally inoculated noted that an intermediate pyrexia occurred which lasted for 3 weeks the trypanosomes appearing in the blood on the 38th day. There were no other striking clinical manifestations.

Muhleus and his associates (1925) inoculated 6 individuals with general paralysis with *T. cruzi*, of whom only 3 became infected. Subsequently F. V. Chagas (1934-36) inoculated with the parasite a number of patients suffering from malignant disease. In these cases also the symptoms were mild.

The incubation period in man has been given as between 7 and 14 days. E. V. Chagas (1935) found that the incubation period in human beings that he experimentally infected was from 10 to 12 days.\*

Both acute and chronic types of the disease have been observed.

**Acute Type**—This form of the disease has usually been reported in children during their first year or two of life. It may be attended by a



FIG. 51.—Courtesy of Dr. S. Mazza.

high continued fever which may show a slight morning drop. There is often marked puffiness of the face and in Brazil enlargement of the thyroid has often been associated. A combination of oedema of the face and conjunctivitis is very suggestive of the disease. The oedema may be so marked that the eye cannot be opened. It is usually unilateral and may be due to the bite of the infected bug as it is assumed that the face and eyelids or conjunctiva constitute the usual portals of entry of the virus. The oedema may spread widely from the face over the body. Chagas attached great importance to it as a frequent early sign in acute cases of the disease. He noted that when the flagellates disappeared from

In Packham's experimental case the onset of illness was 10 weeks after infection. Parasites were found in the blood from the 21st to the 63rd day. Symptoms were mild.

the blood and the temperature became normal the oedema as a rule subsided but that in severe cases it might persist for an indefinite period. It has been described as a hard oedema of elastic consistency which does not pit on pressure. Chagas thought it was due to the myxoedematous infiltration of the subcutaneous tissue and was explained by a specific action of the parasite or its toxins on the thyroid gland. Oedema in Chagas's disease has also been noted by Escomel in Peru, Tejera in Venezuela and Reichenow in Guatemala.

*Ocular Symptoms*—Romano (1935) called attention to unilateral ophthalmia characterized by palpebral oedema, conjunctivitis and swelling of the regional nodes. He thought the condition of diagnostic significance and that the ophthalmia was due to a local inoculation of the conjunctiva with the parasite introduced in the faeces of the *Triatoma*. He reproduced the condition experimentally in the monkey in this way.

Olle (1937) and Mazza (1938) have also emphasized the importance of ocular symptoms in the disease in which there is uni- or bilateral palpebral oedema and conjunctivitis. In some cases Mazza observed disturbances of vision and exophthalmos. Chagas also has described ophthalmitis with suppuration. Mazza has found leishmaniforms in sparse granulations over the lower tarsal conjunctiva together with giant cells. In several of his cases dacryoadenitis was prominent in one spreading to the submaxillary and cervical glands.

Other lymphatic glands and the spleen also may be enlarged. The case may give the picture of a meningitis in which form the disease is exceedingly fatal. During the febrile period parasites are to be found in the blood but in the afebrile interval which alternate with the febrile ones parasites are absent or scarce.

Mazza and Urcelay (1941) have reported upon cutaneous lesions of Chagas disease (Chagoma) which may be produced by the injections of filtered emulsion of the disintegrated bodies of *T. cruzi* taken from culture. Pathologically there is a fat necrosis especially of the tissue cells. It is an initial inflammatory stage with proliferation of the reticular histiocytes. The primary tumor may appear at the bite of the *Triatoma* and secondary tumors develop elsewhere.

*Chronic Type*—If a child does not die or recover from the acute stage the disease passes into a chronic one where in addition to enlargement of the thyroid and the lymphatic glands, loss of hair, dullness, apathy, nervous disorders, alterations of speech and particularly convulsions are said to be striking symptoms. The type of the disease as seen in adults is generally chronic. In the chronic cases the parasite is no longer found in the peripheral circulation but is presumed to be present in the tissues. The adults were said to often show enlargement of the thyroid gland and manifestations of myxoedema. The lymphatic glands may be particularly attacked but in other instances the adrenal has been involved and then symptoms of Addison's disease appeared. If the heart is involved cardiac irregularity may be striking. There may be an irregular fever accompanying the symptoms and a marked anaemia.

Chagas also described in detail in earlier years a cardiac form of the disease in which the parasite invaded the myocardium. He divided the cases into 2 groups: one in which the cardiac changes were of muscular origin and the other in which the changes were associated with deficient nervous influence. The latter however were usually associated with the former. Arrhythmia constituted the most important feature in such cardiopathies and its various types indicated the anomalies of the principal functions of the muscles. The properties of the cardiac muscle fiber that became principally affected were those of excitability and conductivity. The alterations of excitability

included extrasystoles which occur here with extreme frequency and with great variety. The extrasystoles were of auricular or ventricular origin. They may be repeated in each cardiac cycle giving to the pulse the character of bigeminy. Alterations of rhythm may be observed in any age even in children of 6 and 8 years. Next to the arrhythmia from extrasystoles attributable to disturbances of excitability came in order of frequency the alterations of the conductivity of the myocardium and all grades of disturbances of the function may be present from its slightest grades up to complete block with interdependence of the sino auricular and ventricular rhythms. When the bundle of His was attacked by the parasites he found there might result complete heart block the true Adams Stokes syndrome in which the concomitant nervous disturbances are present.

Chagas believed that there is no other disease in which the slow pulse was observed with so great frequency. Heart block may occur in children of 8 to 12 years. Death caused by the cardiac form usually occurred from asystole due to progressive weakening of the heart. The patients then presented generalized and progressive oedema, visceral congestion and other symptoms that characterized cardiac asystole. Another mode of death was from cardiac syncope individuals in conditions of relative health dying suddenly. Chagas believed that these were either cases of complete heart block or death was due to ventricular fibrillation. Thus the patients frequently complained of precordial anxiety and a sense of constriction other patients referred to general malaise with unpleasant perception of the heart beats finally a large number of patients complained only of the agony without being able to define or localize the sensations that constitute it. Palpitations and faintness were also very common symptoms. Faintness at other times might be intense and accompanied by vertigo and loss of consciousness. With reference to prognosis the cardiac form was the type which occasioned the greatest mortality the disease proceeding more or less rapidly to a fatal termination.

Couto (1936) has also reported in a somewhat similar manner regarding the cardiac disturbances associated with myocardial lesions emphasizing the cardiac arrhythmias the bradycardia and occasional extrasystoles. In one case the symptoms of heart block became especially manifest and Stokes Adams syndrome appeared.

During recent years there have been great differences of opinion regarding the symptoms originally reported for this disease. Kraus emphasizes that the symptomatology of the chronic form had been confused owing to the fact that many patients were suffering from endemic goitre and cretinism and points out that Chagas's work was done in a hilly region in which 75 per cent of the native inhabitants have goitre and where a cretin or paralytic occurs in every family. A study of the prevalence of endemic goitre in Brazil in four of the northern provinces other than Minas Gerais (1938) showed that from 15 to 45 per cent of the inhabitants were suffering from goitre. Kraus and others appear to have demonstrated that goitre cretinism idiocy aphasia and infantilism are not the result of infection with *T. cruzi*, but are manifestations of an entirely independent condition namely endemic goitre and cretinism. These observations are also borne out by the experiences in Guatemala and Panama where none of the cases showed evidences of goitre or of cretinism. In the report of De Coursey's fatal cases in Panama the thyroid was found to be firm and of normal size hence it seems that in many of Chagas's original cases the trypanosomal infection was superimposed upon the goitre. Leite (1939) has studied the question anew in Brazil and brings further evidence

that the occurrence of goitre in patients suffering from American trypanosomiasis cannot be considered as an essential part of the disease and is probably accidental

The question of whether *Trypanosoma cruzi* is responsible for the large amount of chronic myocarditis which prevails for example in Brazil is still disputed. At the present time it can only be stated that there seems to be fairly clear evidence that myocardial degeneration is a very common cause of death in the regions in South America where infected *Triatomata* and cases of human infection with *T. cruzi* are known to occur but the evidence that such myocardial degeneration is associated with previous infection of *T. cruzi* is by no means satisfactory since this form of chronic heart disease may be due to myocarditis of other origin as to syphilis for example. Certain of these cardiac disturbances may possibly have their origin in or be influenced by vitamin deficiencies. Mazza (1938) reported a chronic case of the disease in a child detected when 6 years old who was seen at intervals up to the time of her death 10 years and 5 months later. At the autopsy there was chronic infiltrative myocarditis with cellular infiltration degeneration or striation in places. Unfortunately there is no report of whether trypanosomes were found at any stage of the disease.

### PROGNOSIS

The acute stage of the disease is usually of short duration. Among very young children in severe cases a considerable proportion of deaths have been recorded by Chagas. In 1916 he studied 29 acute cases. Of these 15 were in the first year of life, 11 in the second and one of 3 and two of 4 years. Eleven of the patients died, 8 of whom were under 1 year of age. Of 19 cases discovered in Panama, 7 were under 3 years of age and 3 of them died. Two of the 3 acute cases which have been reported in Venezuela died, both of which were in children. Of the 83 cases diagnosed by the discovery of the parasite in Argentina, 26 were under 3 years of age and 4 of the 5 cases recorded as having died were in this group. Yorke (1937) reports that among the 117 cases including some adults which have been diagnosed by discovery of the parasite in places other than Minas Geraes (4 from Sao Paulo and 113 from countries other than Brazil) only 7 deaths have been recorded. In these 7 apparently the diagnosis was definite while Mazza (1937) who has analyzed reports of 240 cases occurring in the Argentine states that the fatality rate was only 5.8 per cent. Talice (1938) describes an acute case in a man 20 years of age whose blood was positive in which the duration of the disease was only 10 days and the patient discharged from the hospital well.

Chagas believed that spontaneous cure does not occur but that those who escape death in the acute period all pass on to the chronic stage of the disease the manifestations of which are due to the multiplication of the parasite in the internal organs. It has already been noted that in older children and adults the infection frequently has produced no important symptoms beyond possibly a mild febrile disturbance. The outlook



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strictly specific. The value of the antigen is said to be directly proportional to the degree of parasitic infestation of the organs from which it was made.

Lacorte in 1927 used this complement fixation test in 200 suspected cases of Chagas's disease in Minas Geraes. In 159 (79.5 per cent) the reaction was positive. Of these cases only 17 per cent gave a positive Wassermann reaction and these showed syphilitic lesions. Villela (1930) reported upon the Machado test with the serum of 186 patients and obtained positive results in 29 per cent. In 83 of the 186 cases the Wassermann reaction was also performed with 17 positive result but there was no parallelism between the 2 reactions. Dias and Mazza (1934) have also found a positive Machado reaction in several undoubted cases. E. V. Chagas in 1934 reported a positive Machado reaction in a case of malignant disease which he infected experimentally with *T. cruzi*. Before the infection the Machado reaction was negative but as early as 10 days after the inoculation the serum gave a positive reaction which

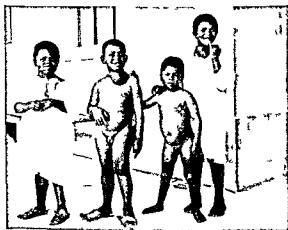


FIG. 5.—Cases of chronic *Schistosomiasis* infected with gonorrhea and chlamydia. Hospital of the Oswaldo Cruz Institute, Rio de Janeiro. (After Muhlens.)

became strongly positive on the 26th day. The patient lived for 6 months after the experimental infection and during the whole of the time the Machado reaction remained strongly positive. He also remarks that individuals who have been removed from an endemic area and who have not been exposed to reinfection may continue to give a positive Machado reaction for at least 15 years.

Kelser (1936) described a modification of the complement fixation test. In previous work the antigens employed had been prepared from organs of laboratory animals artificially infected with *T. cruzi*; hence the potency of the antigens made from them differed markedly. Kelser prepared his antigen from artificial cultures of *T. cruzi* in blood dextrose agar media. With this antigen he tested over 400 serum specimens including a number of known cases of Chagas's disease in man and lower animals. The test appeared positive in all known cases of the disease and negative when there was no evidence of Chagas's disease. Clark (1938)

in chronic cases associated with endemic advanced goitre and cretinism is still further complicated

### DIAGNOSIS

Definite diagnosis depends upon the demonstration in the blood or tissues of *T. cruzi* or the demonstration of this trypanosome in animals inoculated with the blood of a patient. It should be emphasized that frequently it is only during the acute febrile stages in children, or in febrile attacks during the chronic stage in adults that the trypanosomes may be discovered in the circulating blood. In the early acute stage of the disease, the microscopical examination of fresh cover slip preparations or of stained films may suffice for their discovery. Sometimes however for their detection it may be necessary to centrifugalize the blood to concentrate them. When the parasites are not found in this way inoculation of the blood into susceptible animals has been found to be of great value. From 5 to 10 cc of blood may be injected into guinea pigs or puppies. Usually after about 2 weeks they are found in the blood of the animal. If the trypanosomes are not subsequently found in the blood of these animals one may examine sections of muscle of the animals for the presence of the leishmania forms and attempts for cultivation from the animal's blood on NNN media may also be made. A number of investigators have been able to demonstrate the presence of trypanosomes in the blood of inoculated animals when they were too scanty to be recognized by direct examination of the patients blood. Mazza (1939) suggested the examination of sections of the liver or of the heart muscle obtained by the viscerotome when an autopsy is not obtained. He thinks a form of nodular hepatitis is characteristic.

Brumpt has advocated the xenodiagnostic method in which laboratory bred *Triatoma* are allowed to bite the individual suspected of having the disease. After about 2 weeks the intestinal tract of the bugs is examined for parasites. Great care must be exercised in the application of this test to be sure that the *Triatoma* are primarily free from infection and this is not always simple. Also, it has been established that bugs can infect one another by coprophagy. Dias in Rio de Janeiro, reported in 1936 that he had applied the test in 43 cases with 3 positive results. In 1939 he reported 2 further cases, in which this test was positive 16 years after the original infection and when the presence of *T. cruzi* could no longer be demonstrated either by direct examination or by inoculation into guinea pigs. In his opinion, it is a diagnostic method of value. Torrealla (1941) has diagnosed 22 cases in Venezuela by this method.

*T. cruzi* has usually not been found in the lymphatic glands but the trypanosomes have in some instances been reported in the spinal fluid in severe cases with meningo encephalitis. Biopsy of an infected muscle has also been employed for diagnosis. Positive results by the above methods have been obtained in only about a third of the cases.

Villela and Bichlao attach value to the Machado reaction described by Guerrero and Machado (1913). They believe that a glycerin and water extract of the heart and spleen of heavily infected puppies as antigen is

strictly specific. The value of the antigen is said to be directly proportional to the degree of parasitic infestation of the organs from which it was made.

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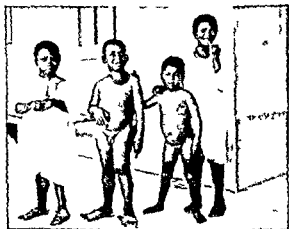


FIG. 52.—Cases of chronic Schistosomum infection with gonorrhea and erythema at the hospital of the Oswaldo Cruz Institute, Rio de Janeiro. (After Muhlstein.)

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reports that this complement fixation reaction is still being employed in Panama and the total number of positive cases so far is 62

It should be borne in mind that a considerable number of cases of visceral leishmaniasis have recently been detected in Brazil and care must be taken to differentiate this infection from Chagas's disease Clark (1938) says that the serum of several cases of cutaneous leishmaniasis did not give a positive reaction with Chagas's antigen

Craig (1932) gives full descriptions of the Kelsor-Cuerreiro-Machado-Villela and Ricah's Complement fixation tests Lackchanian (1940) has described an agglutination test which he reports gives a high degree of agglutination with the serum of animals infected with *Trypanosoma cruzi*: the immune serum being obtained by inoculating rabbits with washed cultures of this organism This test has apparently not been employed for the diagnosis of human cases Senekjic (1933) has also recommended a slide agglutination test

Mayer and Iñaro (1941) have described the preparation of Cruzin prepared from *T. cruzi* They have performed intradermal tests and have obtained definite positive results in infected persons the test attaining a maximum in 48 hours They report it appears to be of great use in diagnosis Mazza et al (1944) have also used a similar substance of emulsified cultures filtered through paper and a Berkefeld V candle for intradermal reactions injecting 0.1 cc In infected cases the immediate result is a papule and in half an hour a surrounding erythema 1.5 cm in diameter and in 24 hours 2 by 4 cm then fading to the original size by the fifth day

### PROPHYLAXIS

On account of the general poverty of individuals who become afflicted with this disease the prevention of infection among them is difficult In the adobe and thatched huts where the *Triatomata* most commonly hide especially in the grass walls and in cracks and crevices in the adobe walls and roofs screening of the houses would largely be ineffective and sulphur fumigation would probably generally be of little avail The use of mosquito nets on the other hand should be effective to protect the sleeping individual from bites of the *Triatoma* Mazza (1937) in most of the dwellings occupied by patients in the Argentine found puppies and cats naturally infected Such infected domestic animals should of course be destroyed Whenever possible infected houses should also be destroyed and new ones built so that the armadillo cannot burrow beneath them Some of the species of *Triatoma* which normally feed on the armadillo are frequently found in the burrows of rodents and the parasite has been found in these bugs at considerable distances from human habitation On the other hand *Triatoma infestans* is reported by Talice (1938) as being strictly domestic and so never found far from human habitations this species feeding especially on man and domestic animals

### TREATMENT

The treatment so far has been exceedingly unsatisfactory and the drugs which have been found most favorable for the treatment of African trypanosomiasis have been reported as of no value in the treatment of Chagas's disease

A number of new chemotherapeutical drugs of the four amino quinoline series have been recently prepared Of these Iensch (1937)

states that surfen and surfen C and a third substance (related to these two in which the 2 amino methyl quinoline nuclei are joined by diallyl malonyl) possess activity against *T. cruzi*. However these drugs are poisonous. Surfen C certainly is contraindicated in human trypanosomiasis as it produces acute nephritis. Even when used in cattle trypanosomiasis it sometimes produces disastrous results the animals dying within 15 minutes.

King, Lourie and Yorke (1938) in their studies of other new trypanocidal substances found that while undecane diamidine was trypanocidal to some trypanosomes *in vitro* it was without action on *T. cruzi* infections in mice.

Mazza (1940) has reported that a preparation of Bayer 760 has a definite therapeutic activity in the treatment of the disease and that it can be considered as a specific remedy. He found that atebryn which had been advised by some authorities was of no value.

According to Salvador Mazza (1942) Bayer 7602 belongs to the Surfene series of powerful antiseptics. He suggests it owes its specific anti-trypanosomic action to the position—4—of its aminoquinolyl group. The Severkusen Laboratories which produce Bayer (AC) 7602 have not disclosed their formula.\* Other observers among them Cardoso and Rosenfeld have not obtained favorable results with this drug. However Mazza feels that this was probably due to the small doses of the drug which were used and to the protracted interval between doses. He believes that the dose should reach a level of 100 mgm per kilogram of body weight in the shortest possible time and that this dose should sometimes be surpassed if there are no contra-indications. It has already been noted that drugs of the Surfene series have been shown to be poisonous and in some instances to have caused nephritis†.

Kofoid (1937) has found that in cultures of *T. cruzi* arsenious tri-thiosalicylic is most toxic for this parasite. However this substance is also highly toxic to mammals.

Culbertson and Kolodny have shown that rats which have recovered from *T. cruzi* infection are completely immune to reinfection. They found that administration of serum of a recovered animal will not prevent prophylactically but will reduce the severity of the infection. When infection was established this serum would reduce the number of trypanosomes in the blood but the parasites increased again when the immune serum was eliminated.

When Chagas's disease is complicated with symptoms of myxedema treatment with thyroid extract has been advised.

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This product has now been analyzed and manufactured by the Imperial Chemical Industries Limited. Fulton (1943) has found that while the drug in experimental animals when injected had a definite action in freeing the peripheral blood of parasites in no case was cure of an infection obtained. Oral administration had little or no value. Only small ineffective doses were tolerated intravenously.

† Mazza now recommends (1943) Bayer 9736 (As) as less poisonous.

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## Chapter V

### THE LEISHMANIASES

Under the term leishmaniasis there have been included three affections known as Indian kala azar infantile leishmaniasis and tropical or oriental sore conditions caused by protozoan parasites perhaps of different species or varieties of a species of the genus *Leishmania* although morphologically the organisms are practically identical It is now believed that Indian kala azar and infantile leishmaniasis are either closely related or identical diseases and that the naso oral South American leishmaniasis is a variety of oriental sore The clinical forms may be conveniently divided into *visceral* and *cutaneous* leishmaniasis The *visceral* forms are characterized by irregular fever of long duration a chronic course splenic and often hepatic enlargement emaciation anaemia and leukopenia In the *cutaneous* forms nodules and ulcerations result and the infection with the parasite is usually local and not general The skin and the exposed parts of the body and in the South American form the mucous membranes of the nose mouth and pharynx also are particularly attacked In addition dermal leishmaniasis may occur as a complication or sequel to visceral kala azar and particularly as a post kala azar condition in cases which have been treated with antimony compounds

**Synonyms**—For Indian Kala azar—dumdum fever tropical splenomegaly black sickness, for infantile kala azar—splenic anaemia of infants ponos for Eastern cutaneous leishmaniasis—oriental sore Delhi boil Biskra button Bagdad boil bouton d'Orient Aleppo boil granuloma endemicum salek (Persia) for American cutaneous leishmaniasis—espundia bubas uta forest jaws

**Definition**—Kala azar is an infectious disease characterized by a persistent fever of alternating remittent or intermittent type The disease rapidly leads to a cachectic condition with ultimate great enlargement of the spleen and later of the liver There is frequently extreme emaciation The fever lasts from a few months to several years The course of the disease is often concluded by a terminal infection the mortality formerly averaging in India in untreated cases as high as from 80 to 96 per cent The malady is due to a minute protozoan *Leishmania donovani* which has been shown by artificial cultivation to be one stage of a flagellate parasite and the most recent evidence indicates that it is transmitted probably by a blood sucking arthropod *Phlebotomus*

**Geographical Distribution and Prevalence**—The disease is widespread and very prevalent in parts of Asia and in Europe in countries bordering



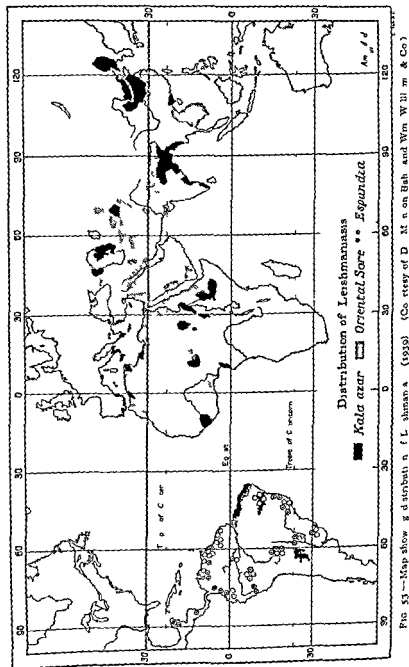


FIG. 53.—Map showing distribution of *Leishmania* (1939) (Courtesy of Dr. M. A. On Ush and Wm. W. H. m. & Co.)

on the Mediterranean and in parts of Africa. In India where it was first recognized its incidence while largely limited to the eastern side of the country as far south as Tuticorin has gradually been found to be wider than was first supposed. Assam, Bengal, Bihar and Madras still remain among the most heavily infected centers and there has been an increased incidence in these areas and in Sikkim in recent years (Napier, 1939).

Shortt, Craighead and Swaminath suggest that an extension of the endemic areas has probably occurred to include not only the whole east coast of India including the southern portion of Orissa but that also there has probably been an extension of the Bihar focus in a westerly direction to Lucknow and Allahabad. However the disease in some of the western areas is not common and other observers have thought that the cases seen in the Punjab for example were largely imported ones. In the north its extension in Assam and Bengal is limited by the foot hills of the Himalayas and in the east apparently by the range of mountains that divides Bengal from Burma.

Kala azar is endemic in every district in Bengal. Napier estimated that there were about a million people affected with it in this province and that in the Out Patient Department of the Calcutta School of Tropical Medicine alone more than 1000 patients were treated in a year. In Assam almost the whole of the Brahmaputra Valley has been infected. Phipson (1939) states that the disease has nowhere been completely stamped out in this area and is still found in all the plains districts with the exception of the most northerly district of L. L. L. The medical officers of the Assam Bengal Railway found the disease common in the plains portions of the railway but rare in all portions of the hill section between Sylhet and the Brahmaputra Valley and absent from the village above 2000 feet. However reports in 1939 show that a few cases have occurred in the Garo Hills up to an altitude of 4000 feet.

With reference to its prevalence in Assam during the epidemic (1944-1946) Shortt, Craighead and Swaminath report that the number of cases treated during 1944, 1945 and 1946 was 48,770, 60,940 and 46,231. However during the past seven years Napier and Phipson (1939) give the average number of cases treated as 2000. Nevertheless Phipson (1939) believes that the conditions regarding kala azar in Assam are unsatisfactory because although the incidence has been reduced from 7.68 per mille at the height of the epidemic to about 1.5 per mille and remains practically stationary for the past 7 years during the current year the incidence in most districts has shown a significant and perhaps an even ominous rise. In the Province of Madras the crowded native Indian quarters in Madras City and its suburbs have long been important foci of infection. The whole eastern portion of the Madras Presidency south of a point a little north of Madras City is also endemically infected. Napier (1939) emphasizes the increased incidence between Bihar and Bengal. Kala azar cases coming for treatment in Bihar in 1936 were roughly 1000 in 1936, 15000 in 1937, 5000 in 1937, no less than 91000. These cases were only those that came voluntarily for treatment. It is assumed that about an equal number of cases existed in the villages and did not come to hospitals or dispensaries for treatment. In Bengal excluding cases treated by hospitals and special kala azar dispensaries in 1936 some 120,000 cases came for treatment in 1936, some 160,000 and in 1937 some 200,000. Castellani has seen kala azar in Ceylon and has noted its occurrence in Burma but Napier does not consider it endemic in either of these localities.

In China it has been found to prevail from Peking in the north through Chihli, Shantung, Kiangsu and Anhwei in the region of the Yangtze River as far south as Canton. According to Taylor it prevails widely through south Manchuria. As is the case in India the disease in China is confined to the low lying alluvial plains usually below an elevation of 200 meters. The western and northern limits are so far as is known the hills bounding the coastal plain. Young and Hertig found

the most heavily infected area to be a belt in the region of the old course of the Yellow River from the Province of Haichow to the sea with the heaviest centers near the Grand Canal. The incidence decreased on either side of this belt, though there were small scattered foci of the disease chiefly in Shantung.

In earlier years Castellani reported the presence of the disease in Indo China and Rogers in Siam. Smits has reported its presence in Sumatra but this last observation has not apparently been confirmed anywhere in this portion of the world. The disease has not been observed in the Philippine Islands.

In Central Asia Artamonoff found centers of infection along the Central Asian Railway and especially in Tashkent. It extends laterally from the great centers of Samarkand, Kokand and Andizhan in which it occurs chiefly in the sectors of the railway stations and more frequently amongst Europeans than among natives. Of 314 cases seen by Artamonoff only 100 were in natives. The disease is also endemic in Arabia and Syria (Lepine, Hietel) as well as in Palestine (Canaan). Kulz has reported its presence in lower Mesopotamia.

In Europe the affection has been found in southern Russia in Transcaucasia, and extending eastward into Turkestan in Asia. The Mediterranean littoral contains several endemic centers—in Greece in the southern sections of Italy, France and Spain and in the islands off the coasts of these countries. The 3 largest foci according to Adler and Theodor (1931) are Catania, Naples and Palermo. A few cases have been reported in northern Italy and northern Spain. DeMoullae (1939) reports that it is becoming increasingly common in Cherbourg. Kırım lidis (1939) has reported kala azar wide spread in the northern half of the province of Argolis, Greece and Arar has reported a few cases, from 1931-1938 in Turkey.

In earlier years, the disease in the south of Europe bordering upon the Mediterranean was supposed to be confined to children. Recently numerous cases in adults have been encountered in southern Europe.

In Africa the disease occurs in North Africa (Morocco, Algeria, Tunis, Tripolitania, Cyrenaica and Egypt). Cyrenaica appears to be the least affected though nowhere in North Africa does kala azar appear to be as prevalent as in certain other parts of the Mediterranean region.

It has been encountered in the Kassala and Blue Nile districts west of Abyssinia from Khartoum in the north to Kodok in the south. The incidence of the disease is reported as increasing in the Sudan especially along the Entreat and Abyssinian frontiers where 328 cases were reported in 1938. Its presence has also been noted in Kenya Colony and near Lake Chad and in the Gabon (French Equatorial Africa). Owen (1930) in reporting a case from Kano, Nigeria emphasized that the disease is uncommon in West Africa. Stephenson (1941) has reported an epidemic of Kala Azar in the Sudan which began in 1932 and lasted for about 8 years. In 3 years at least 300 cases occurred in a population of 8 000 and the fatality rate was 80%. Few recovered even among those admitted to hospital and given the standard treatment.

The disease was not reported in the Western Hemisphere until recently. Mignone apparently first observed a case in Asuncion, Paraguay. Mazza and Arias reported 2 cases of infantile kala azar in the northern part of Argentina where there were many immigrants from countries where kala azar existed. In 1934 Penna reported infection of the liver with *Leishmania* in several individuals in Brazil. More recently Chagas

(1936) in examination of specimens of the liver obtained by the viscerotome in the diagnosis of yellow fever found *Leishmania* in 41 of 47 000 specimens. Later Penna reported the number of infections was increased to 85. The distribution was found to be fairly general in the northern and eastern district of Brazil and in the Chaco district of the Argentine. Cases of infection were observed in individuals varying from 45 days to 56 years of age the highest incidence of infection being under 6 years of age. Cases have also been reported from Bolivia.

Imported cases of the disease have occasionally been observed and reported in the United States. One such case diagnosed by spleen puncture occurring in a Chinese student was reported by Mathieson and Watson in Minnesota in 1939.

### VISCERAL LEISHMANIASIS

**History**—The early history of kala azar in India is somewhat obscure. The disease first attracted public attention in 1882 when Clark of the Sanitary Commission of India gave an account of 100 cases described as a severe form of malarial cachexia depopulating certain areas at the foot of the Garo Hills Assam. The Garos called the affection kala azar (black fever) and it appears that it had been known to them since about 1869. Its occurrence among them was one reason for their failure to be able to pay the land rents owing to the amount of sickness and disability it occasioned in the tribe. The disease at times terrorized the natives who are said often to have intoxicated afflicted patients and burned them in their huts to eradicate the malady.

This epidemic of fever advanced slowly up the Assam Valley and by 1889 had spread 150 miles up the valley of the Kamrup district.

It was also known that from 1854 to 1875 epidemics of the affection under the name of Burdwan fever occurred in lower Bengal occasioning a quarter of a million deaths and in some districts as many as 50 000 people succumbed. While the nature of Burdwan fever must be regarded as obscure it seems reasonably certain that the disease which was described by Dr French in 1872 as having existed in the Burdwan district since 1862 was kala azar.

The Assam epidemic maintained a steady rate of progress up the valley and by 1896 it had overrun the next most easterly district of Nowgong the death rate being so considerable that there was a decline of 31.5 per cent in the population of Nowgong in that decade. Napier reported that the disease was at its worst in Nowgong between 1890 and 1900 and during this period the population showed a 25 per cent decrease. He suggests that in the great Assam epidemic virgin soil was being invaded whereas in South Bengal it had been endemic for many years.

The early history of kala azar in China is unknown. No great epidemic in China comparable to that of Assam has been recorded though mild outbreaks extending over several years at a time and outbreaks occurring in cycles of 15 or 20 years are said by Young and Hertig to have occurred. Cochran who investigated the subject in 1913 found that all the earlier authentic cases had occurred in the country north of the

Yangtze River with the exception of Wuchang and Hankiang which are on this river

Our knowledge of Mediterranean leishmaniasis or kala azar probably goes back to 1835 in which year Roser directed attention to the occurrence of *ponos* or a painful enlargement of the spleen in young children on the Island of Spezzia. Pallas also shortly afterward referred to the condition and it was learned that it was endemic in the Island of Hydra as well as in Spezzia.

Karamitsas (1880) and Stephanos (1881) gave excellent clinical accounts of the affection which was said to commence during the first year of life the earliest symptoms being languor and pallor fever of an irregular character and enlargement of the spleen. Emaciation became progressive the digestion was enfeebled and constipation was nearly always present. The spleen gradually attained a great size and was tender these symptoms determined the name *ponos*.

Pathological examination showed the characteristic lesions of tuberculosis leukaemia and malaria as absent. Gabbri later proved that this disease *ponos* as seen in Spezzia was a form of leishmaniasis. However the parasitic causation of Mediterranean kala azar had already been demonstrated in 1905 by Pianese.

### ETIOLOGY

Giles who studied the affection known as kala azar in 1887 having found ova of the hookworm in a great majority of the cases concluded that the disease was undoubtedly a form of ankylostomiasis associated with malaria. Rogers in 1896 after studying kala azar concluded that it was an intense form of malarial fever. This theory was opposed by Manson but was agreed to by Ross in 1899 who thought however that there might be in addition some other form of infection. In 1902 Bentley studied the disease and from the positive agglutinating reactions he obtained concluded that it was a malignant form of Malta fever. In 1903 Manson suggested that it might be caused by a trypanosome since the absence of malarial parasites and failure of treatment by quinine argued against its being of malarial origin. On May 30 1903 Leishman reported finding what he considered a degenerated form of a trypanosome in the spleen pulp of a soldier who died in 1900 at Netley of dum-dum fever. He first saw these bodies 2½ years before he made his publication noting them at the time of making an autopsy but he was then at a loss to explain their significance. However in 1903 after examining a rat infected with trypanosomiasis he came to the conclusion that there was a similarity in the parasites in the human spleen and those in the rat. In July 1903 Donovan in Madras reported the finding of similar parasites in specimens made from splenic puncture of cases of dum-dum fever taken during life. Laveran and Mesnil then examined specimens of Donovan's films and apparently owing to the fact that a number of the parasites appeared associated with or adherent to the red blood corpuscles regarded them as piroplasmata and proposed the name of *Piroplasma donovani*. However on further study this view was abandoned by them. Ross (Nov. 14 and 28 1903) regarded the parasite as a sporozoon and suggested the name of *Leishmania*. Thus the accepted name of the parasite became *Leishmania donovani*.

In regard to the etiology of cutaneous leishmaniasis Wright in 1903 in the study of a case of tropical ulcer in Boston which occurred in a child from Armenia found certain bodies which bore a close resemblance to those described by Leishman and Donovan. He proposed the name of *Helcosoma tropicum* suggesting that the parasite was a protozoon and allied to the microsporida. Marznowsky and Bogrow (1904) working in Russia also found a similar organism in a case of oriental sore in the body of a boy who had resided in Persia and proposed the name *Ovoplasma orientale* for the parasite. Subsequent investigators have shown that both Wright's and Marznowsky's organisms are morphologically indistinguishable from that of kala azar and must be included in the same genus. Hence the correct name for the parasite of oriental sore is *Leishmania tropica* Wright 1903.

Because of the light thrown upon the etiology by these investigations it seems not improbable that this parasite of cutaneous leishmaniasis had been seen previously by Cunningham in 1885 in the examination of Delhi sore although he expressed the opinion that it was impossible to come to a definite conclusion as to their nature or to the relation which they bore to the disease. When one examines Cunningham's illustrations one must admit that they do not definitely show that the bodies in question are parasitic in nature.

The etiology of cutaneous leishmaniasis has been discussed here with the visceral form kala azar not on account of any definite clinical relationship that may exist between the two but for the reason that the causative organism evidently belongs to the same genus and is closely related if not identical.

In 1904 Rogers succeeded in cultivating *Leishmania donovani* from human blood obtained by splenic puncture which he placed in 1 cc. of sterile salt solution and 5-10 per cent citrate of soda the cultures being kept at 20-22 C and not above 25 C. He emphasized the fact that the cultures will not grow if bacteria are present. Usually in 3 days the flagellate stage was obtained and the parasites began to multiply.

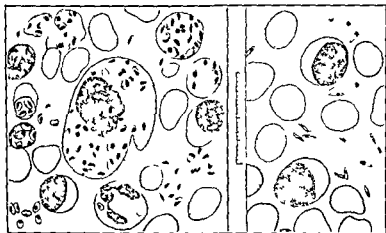


FIG. 54.—Leishman bodies in smear of splenic pulp. On the left small forms in the right boat shape of form. (After R. R. Knowles from Brumpt.)

Four years later (1908) Nicolle and Nicolle and Sicre obtained cultivation of *Leishmania tropica* from oriental sore—an observation which demonstrated more clearly the close relationship of the two parasites and Nicolle gave the name of *Leishmania infantum* to the parasite causing kala azar in the Mediterranean area. Subsequent work performed in different parts of the world has established conclusively the fact that *Leishmania donovani* and *Leishmania tropica* are the actual causes of kala-azar and oriental sore.

Later on cutaneous leishmaniasis was shown to occur in South America and in 1913 the Harvard Commission demonstrated that the disease known for centuries in Peru as uta was in fact a form of leishmaniasis and the members of the Commission recovered and cultivated the leishmania from the lesions. Escamez who studied particularly the nasal-oral form of leishmaniasis termed *espundia* sent preparations from the lesions to Laveran and to Nathan Lerner who demonstrated leishmania therein thus confirming Escamez's view of the nature of the affliction. Vianna had already in 1911 suggested the name of *Leishmania brasiliensis* for the parasite that he, Lindenberg, Splendore and other workers had observed in cutaneous and nasal-oral lesions in Brazil. E. Chagas and his colleagues (1937) have given the name of *L. chagasi* to the parasite obtained by puncture of the liver from individuals in Brazil.

**Classification**—The parasites belong to the family *Trypanosomidae* and the genus *Leishmania* and occur in the leishmanial form in man and in the leptomonas form in the bodies of various insects. From a purely morphological point of view, the members of the genus *Leishmania* are not distinguishable from those of the genus *Leptomonas*. In both, there occur only the leishmania and leptomonas forms. However the members of the genus *Leptomonas* are passed on from one invertebrate to another by the contaminative method by means of encysted forms passed in the faeces. No such stages are known in the case of *Leishmania*.



FIG. 55—Flagellate forms of *L. donovani* from culture. (Courtesy U. S. Army Medical Museum Neg. #3789)

The *Leishmania* (Leishman Donovan bodies) are round or oval bodies averaging  $2-5\mu$  in diameter. The nucleus is relatively large and peripherally placed; the kinetoplast is smaller, rod-shaped or oval and set at a tangent to the nucleus. A short slender filament, the axoneme, is sometimes seen extending from the blepharoplast to the periphery. In a Romanowsky stain the cytoplasm is faintly blue; the nucleus appears as a mass of bright red fine granules, and the kinetoplast is deep reddish purple. One or more vacuoles are common (see Fig. 54).

The *Leishmania* multiply by binary fission within the cells of the host, which may contain as many as 200 parasites in a single cell. Eventually the affected cell becomes destroyed by this proliferation and disintegrates, setting free the *Leishmania*, which are then taken up by other endothelial cells or by the leucocytes and monocytes of the blood.

**Cultivation** may be obtained readily in citrated blood or in moist tubes of NNN medium. Growth occurs in the water of condensation. Incubation must be at a temperature of  $20-24^{\circ}\text{C}$ . Bacterial contamination inhibits their growth.

Cleveland and Collier found the following media most satisfactory

A paste is made of 25 gm. of Difco special haemoglobin in 250 cc of distilled water then an equal amount of Difco liver infusion agar is added to it (removing the agar before use) Then sufficient glucose to make the mixture 1 per cent is added This mixture has also been prepared by the Digestive Ferments Company in a dehydrated form and can be used by dissolving the powder in distilled water tubing and autoclaving This medium has been named haemoglobin liver broth Lwoff (1938) has demonstrated that for growth leishmania require ascorbic acid haematin as well as an unknown substance which is present in the serum Yen and Chung (1938) have employed embryonic chick tissue for cultivation of leishmania In the experiments in which whole chick embryo was minced finely and suspended in Tyrode's solution successful cultivation was obtained However in a media containing either embryonic chick liver or intestine alone or of chick liver and chick brain no growth was obtained

**Leptomonas Forms in Cultures and Insects**—On cultivation the *Leishmania* develop into *leptomonas* forms similar to those found in

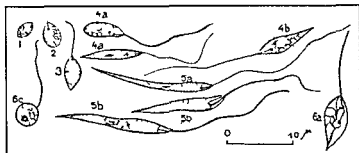


FIG 56—*Leishmania donovani* Cycle of development in culture. 1 Parasit of human body. 2 3 4 4b and 5a Parasit occurring in the first to fifth day of culture. 5b Parasit developing forms as they are detected with the fluid in the Phlebotomus. 6a and 6b Degenerating forms (After Christophers Shortt and Brumpt)

insects After about 48 hours they increase in size and elongate until they reach a size of from 14–20 $\mu$  in length by about 2 $\mu$  in width The kinetoplast is situated at the blunt anterior end and from it a long flagellum arises This is about the length of the body of the organism and there is no undulating membrane Multiplication is by longitudinal fission and aggregations of the parasites in the shape of rosettes with the flagella toward the center may be seen Cultures may be kept for a long time by making transfers every 2 or 3 weeks but their virulence is apt to be lost Wenyon and Nicolle and others have preserved strains in this way for over 15 years

Cultures of the organisms from man have been obtained particularly from the spleen and liver as well as from the blood and bone marrow and Shortt, Swaminath and Sen have reported growing the organism from the centrifuged deposit from the urine of 3 cases of kala azar Napier believes its occurrence in the urine is only accidental dependent on the presence of blood or other leishmania infected cellular deposit in the urine



**Differentiation of Species of *Leishmania***—As regards the various species of *Leishmania* which have been described in man (*Leishmania donovani*, *Leishmania infantum*, *Leishmania tropica*, *Leishmania braziliensis*, *Leishmania chagasi* and "the parasite of dermal leishmanoid") it has generally been admitted that they are morphologically indistinguishable from one another.

**Animal Inoculations**—But little information regarding differentiation has been obtained from animal inoculations. It has been found that *Leishmania donovani* which produces a generalized infection in man, may in some instances give rise to purely cutaneous lesions in animal, as it occasionally does in man, while *Leishmania tropica*, which causes local cutaneous lesions in man, may in some instances produce either a local lesion or a generalized infection in animals.



FIG. 57.—Agglutination rosette of flagellate (*Leptomonas* form) in insect. (Dept. Trop. Med., Harvard Courtey Army Trop. Med.)

Mayer found some strains of *Leishmania donovani* more virulent than one of *Leishmania tropica* in that when strains of the latter were inoculated intraperitoneally in European hamsters (*Cricetus auratus*) only on one occasion was a generalized infection produced. Cultures of each species when inoculated into the skin produced similar local haemorrhagic areas. However Row showed particularly that *Leishmania tropica* can induce a generalized infection when injected into the peritoneal cavity of mice.

Adler and Theodor (1930) infected a human being with cutaneous leishmaniasis inoculating material from a naturally occurring, ornamental sore of a dog from Bagdad; they believe that there is proof of a cutaneous leishmaniasis common to man and dog and that *Leishmania tropica* a naturally occurring parasite of the dog. They found that different strains of *Leishmania tropica* varied in their ability to infect mice. Some human and canine strains were found to be non-infective and others to produce visceral and cutaneous lesions after intraperitoneal inoculation.

As a result of his experiments Gupta believes that very little help can be obtained in the differentiation of *Leishmania tropica* and the organism of dermal leishmanoid from the result of animal inoculation only.

Hindle has attempted to show differentiation of various strains of *Leishmania* from their inoculation into Chinese hamsters *Cricetus griseus* and *Cricetus triton*. The results obtained from the inoculation of an Indian strain direct from one hamster

to another resembled those obtained with the Chinese strain of *kala azar*. On the other hand the results of attempts to infect hamsters with *Leishmania infantum* seemed to show that the hamster was much less susceptible to this strain than to the Chinese and Indian *kala azar* strains of *Leishmania*.

Adler (1938) points out that the old view that the South American species of *Leishmania* could be differentiated by the fact that it would not infect laboratory animals is not tenable since he has infected a Syrian hamster with cultures of this strain. Da Cunha also re-emphasizes that the so called *L. chagasi* cannot be differentiated from *L. infantum* or *L. donovani* and that it can produce infection in hamsters, monkeys and dogs.

**Serological Tests**—Attempts have also been made to differentiate the species by serological tests. However as Wenyon and others have pointed out the use of such tests for the separation of true species of *Leishmania* is of very doubtful value.

Noguchi in 1924 and 1936 employed strains of *Leishmania donovani*, *Leishmania infantum*, *Leishmania tropica* and *Leishmania brasiliensis*. Rabbits were inoculated intravenously with cultures of these strains on 4 occasions at 5 to 7 day intervals. The sera from these animals were then used on the cultures to test their agglutinating power. It was found that in dilutions of 1:10 or even 1:100 the serum of the animal inoculated with *Leishmania donovani* agglutinated this organism and *Leishmania infantum* but not the two others. Similarly the serum from an animal inoculated with *Leishmania tropica* agglutinated this organism alone the same as true of the serum of an animal inoculated with *Leishmania brasiliensis*. If the sera were added to the culture media directly they were specific in changing the character of the growth of the homologous organisms. Hence he concluded that *Leishmania tropica* and *Leishmania brasiliensis* were serologically distinct from each other.

Kluger also found that *Leishmania brasiliensis* and *Leishmania infantum* were immunologically distinct from each other and from *Leishmania tropica*. However Wenyon and Koch who carried out comparative serological tests with cultures of 4 strains of *Leishmania* and 1 strain of *Herpetonas ctenocephali* of the dog flea (which has been regarded as a possible vector of canine leishmaniasis) found that anti-leishmania sera affected equally the different species of *Leishmania* but had no effect upon *Herpetonas ctenocephali*.

Chodulnik, Franchin and Pirami (1930), Laurin (1931) and Zdrodowski and Woskressenski (1931) have also employed serological reactions and particularly the agglutination test for differentiation of the species. Laurin, by using the agglutination test, divided the organisms into 3 types—*Leishmania donovani*, identical or very closely related to *Leishmania infantum*, *Leishmania tropica* and *Leishmania brasiliensis*. However they emphasize that identification of *Leishmania* by serological reactions is not so simple as has been supposed since different strains may vary in these reactions.

Also Ray who produced immune sera by the inoculation of rabbits with large quantities of pure cultures of *Leishmania donovani*, *Leishmania tropica* and *Leishmania infantum* found that the sera readily agglutinated the culture forms but that in most cases they were not specific so that separation of the parasites was not possible by serological tests. Gupta also obtained decisive results and Da Cunha (1938, 1940) was also unable to separate *L. chagasi*, *L. infantum* and *L. donovani* by means of serological tests as had been suggested by previous work. Attempted complement fixation tests also did not give satisfactory results. Da Cunha (1941) who has carefully studied the serum agglutination test concludes finally that it is of no value in separating species of the genus *Leishmania* as all the *Leishmania* which have been kept in cultivation contain a common antigen.

**Rickenberg's Reaction**—Burrows, Chodulnik, Meisels and Balachewa have employed the Rickenberg adhesive reaction for differentiation of the species but this reaction has been found to be more unsatisfactory for differentiation than the agglutination test.

Noguchi, Kluger and Cleveland and Collier (1930) attempted to differentiate the human species by the use of culture media containing various carbohydrates but found differentiation by fermentation tests was not possible.

**Differentiation of *Leptomonas Ctenocephali***—Tyzzer and Walker undertook to determine if possible the generic and specific relationship of *Leishmania infantum* of infantile kala azar and *Leptomonas ctenocephali* parasitic in the gut of the dog flea. They concluded from their investigations that the assumption that the organisms are identical can be definitely excluded on account of the differences noted with respect to morphology, resistance to various temperatures, and ability to multiply in the mammalian body. These factors as well as geographical distribution they think make it appear preferable to consider the organisms as distinct.

**Differentiation in *Phlebotomus***—Adler and Theodor (1927) have studied the behavior of *Leishmania* in *Phlebotomus papatasi*. They infected these sandflies with *Leishmania tropica*, *Leishmania brasiliensis*, and 2 strains of *Leishmania infantum*, by feeding them emulsions of parasites through a membrane of rabbit skin, and found that some of the species differ in their behavior in the fly. Forms from cultures of *Leishmania tropica* ingested by *Phlebotomus papatasi* behaved exactly as *Leishmania tropica* ingested by the insect from an oriental sore; that is, the parasites after multiplying ascend to the pharynx and in a few cases enter the proboscis.

The pathogenicity for man of culture forms of *Leishmania tropica* was increased by passing through a sandfly. Adler and Theodor also tested a number of insect leptomonads, and one from a plant in *Phlebotomus papatasi*. These were found to persist in the stomach for periods up to 13 days, and in some cases to pass to the hind gut. They showed no tendency to ascend to the cardia. The authors believe that it is clear that the development of the insect and plant flagellates in *Phlebotomus papatasi* is an illustration of nonspecific behavior.

Hindle performed experiments in which sandflies were fed artificially on cultures of the parasites in the flagellate stage. The method used was that devised by Hertig in which the sandfly was held in position in a glass tube by a split cork, and the medium fed to the insect by means of a fine glass pipette placed over its proboscis. The results of these experiments suggested that the Indian strain of *Leishmania donovani* does not develop in *Phlebotomus chinensis* and *Phlebotomus mongolensis* with the same readiness as Chinese strains of this parasite. Also that *Phlebotomus chinensis* and *Phlebotomus mongolensis* are not favorable hosts for the development of a strain of *Leishmania infantum*. Some of the *Phlebotomus mongolensis* which were fed on cultures of *Leishmania tropica* became infected and showed flagellate forms free in the stomach as did the flies fed on an Indian strain of kala azar or with *Leishmania infantum*. However, the only flies that ever showed flagellates in the cardia and pharynx were *Phlebotomus chinensis* that had fed on hamsters infected with the Chinese strains of *Leishmania donovani*.

Hindle concluded from his results that although there is a general capacity on the part of *Leishmania* after being ingested by various species of *Phlebotomus* to develop into the flagellate stage, it is only when there is some biological relationship between the two that development proceeds further. When this occurs, the flagellates became attached to the lining

of the gut and especially in the cardia they also invade the pharynx whence they may eventually extend into the proboscis

Thus it will be seen that it has not yet been proved conclusively that *Leishmania donovani* *Leishmania infantum* *Leishmania tropica* and *Leishmania braziliensis* are separate species. There are however epidemiological, pathological and clinical distinctions between the visceral and the cutaneous forms of leishmaniasis which warrant the consideration of these flagellates as separate types.

**Relationship of Animals to Human Infection**—The only animal which has been found naturally infected to any extent with visceral leishmaniasis is the dog. The infection of the cat with *Leishmania* has been reported in a few instances. In at least two the infection was visceral. The dog, however, has been shown to be very frequently infected with *Leishmania* particularly in southern Europe, the Mediterranean areas and North Africa, and the infection in this animal is often associated with the disease in children. This naturally suggested the idea of the canine origin of human kala azar.

It was also observed that in certain endemic areas of oriental sore dogs commonly contract the disease as in Bagdad, but that in others the canine disease is unknown or very rare as in Palestine. Similarly in the Mediterranean areas dogs and very young children commonly suffer from kala azar. On the other hand in India the disease was not found or was very rare in dogs and rarely seen in very young children. However the idea of the canine origin of Mediterranean infantile kala-azar was at first much strengthened by the fact that Basile reported finding leishmania like flagellates in dog fleas and brought forward other evidence incriminating this insect as a means of infection although this method of transmission was not later confirmed.

The natural disease in dogs as in man may run an acute or chronic course in which there is loss of weight, fever, anaemia and enlargement of the liver or spleen. The animals may die of intercurrent infections but recovery takes place more frequently than in human beings. The first observation of canine kala azar due to *L. caninum* was made by Nicolle and Compté (1908) in Tunis, an endemic center of infantile kala azar. The rate of infection has been found to vary greatly in the different localities in some instances only from 4-5 per cent of the dogs were infected but in Sicily Basile found 27 infected out of 33 examined. On the other hand Donovan working in Madras examined 1150 dogs, 256 of which came from the portion of the city where kala-azar was abundant without finding a single infection. Donovan and Patton after a further examination of 2000 dogs in Madras and Mackie in Assam also obtained only negative results. Nevertheless the Indian virus may be inoculated into dogs if sufficiently large doses are injected intraperitoneally.

Hence it was doubtful at first whether the naturally occurring disease of dogs was due to *Leishmania donovani* or to some other species but Wenyon pointed out that the frequent association of the disease in dogs with human cases in the Mediterranean area and the morphological identity of the parasites are facts which make it impossible to regard the organism from dogs as other than *Leishmania donovani*. Since the Indian disease is inoculable into dogs he considers the various systemic diseases in man and dogs as due to *Leishmania donovani*. In many instances there has been evidence that the infection has passed from dog to man although many other cases obviously have occurred which cannot be associated with any infected dog.

Basile in Bordonaro, Sicily where there is a high percentage of naturally infected dogs, states that the extermination of these animals led to an almost complete disappearance of the human disease. Chodukin after 5 years study on the question of the correlation between human and canine leishmaniasis in Turkestan emphasizes the degree of contact between man and dogs and points out that in a number

of instances actual contact between kala azar cases and diseased dogs could be traced. Out of 43 human cases 33 were known to have been in contact with dogs 16 having been in contact with known infected dogs.

In China natural infection of dogs with *Leishmania* has been reported by Andrews (1935) Lee (1937) and Feng (1939) and Hoeppli (1939). Andrews and Lee found leishmania in the spleen and liver of 3 dogs and Feng and his associates cutaneous lesions in 12 dogs. Although the relationship between the canine and human disease in China is also not entirely clear nevertheless Feng and Chung (1939) have found that *Phlebotomus sergenti* var *mongolensis* and *P. chinensis* fed on 3 naturally infected dogs and 1 laboratory infected dog all became infected and that the rate of infection of the flies was directly related to the degree of infection of the skin of the dogs. However *P. chinensis* appeared to be a much better intermediate host. In this fly pharynx and proboscic infections were observed from the 5th day after the infected feed but in *P. sergenti* infection of these parts of the fly was never found.



FIG 58—Showing massive flagellate infection in Proventriculus of *P. chinensis* fed on Dog 6 days previously. X 850 (Preparation of L. C. Feng and H. L. Chung courtesy of the Chinese Medical Journal)

Chung and Feng (1939) have also found natural infection of *P. chinensis* with leishmania flagellates. Some of the flies caught in the kennel of a dog were found infected with kala azar. *P. chinensis* was found to readily suck blood from dogs.

Sun and Wu and Raynal (1939) also have found *P. chinensis* to be the most widespread species in North China and regard it as the chief if not the only vector in that region. Although transmission by the bite has not been achieved the intraperitoneal injections of crushed infected insects into hamsters easily produces infection.

Dogs have also been successfully infected with material from human leishmaniasis and canine cutaneous leishmaniasis and human visceral kala azar have been found in the same household.

In Brazil Chagas (1938) has found visceral leishmaniasis in 7 dogs and 1 cat as well as in some wild animals in Brazil.

From a large number (400) of postmortem examinations of dogs Chodukin and Soffieff concluded that cutaneous canine leishmaniasis is merely a symptom of a generalized infection. In some with prominent skin lesions parasites were present also in the spleen or bone marrow. The cutaneous lesions in dogs were reported as analogous

to those seen in hamsters inoculated with *L. donovani* or with the dermal leishmanoid of kala azar seen in India

Hence the most important recent work strengthens the opinion that the dog may be the principal reservoir of the disease in many localities though not in others. Adler and Theobald think that the occurrence of kala azar in dogs and infants in the Mediterranean and not in India may be due to the fact that the Mediterranean vectors *P. perniciosus* and *P. major* may infect their victims very frequently by direct inoculation into the skin at the time of biting while the Indian vector *P. argentipes* may less frequently inoculate the parasites by their bites but cause infection by being crushed. Since dogs are not apt to crush flies and infants are not as adept at slapping and crushing them as are adults this may explain their lack of infection by the Indian species.

**Susceptibility of Animals**—Dogs cats monkeys mice rats guinea pigs Chinese and European hamsters and certain species of squirrels have all been shown to be susceptible to infection with *Leishmania* although greatly varying in this respect. However in many instances the inoculation of the animal fails.

Guinea pigs are difficult to infect but have been shown by Laveran and Pettit and by Gupta to be in some instances inoculable. Usually however they are entirely refractory to inoculation. Rabbits are even more insusceptible and no satisfactory results in general infection have been recorded.

Successful inoculation of monkeys *Macacus sinicus cynomolgus* and *rhesus* has been reported by a number of observers. Monkey infected intraperitoneally may die in 1 or 2 months or the disease may run a chronic course ending in recovery. As in the dog the infection shows many irregularities.

Although intraperitoneal or intravenous inoculations succeed more often than subcutaneous ones none of the laboratory animals so far discussed can be considered as suitable or easily susceptible to infection and none of them has therefore been found suitable for diagnostic purposes or for demonstrating the existence of infection in experimental work. When infection does occur in these animals it is usually of slow development and the number of *Leishmania* found is usually small.

By far the most satisfactory animal so far discovered for experimental purposes is the Chinese hamster. Young and Smillie and Brown first reported that the Chinese hamster *Cricetulus griseus* is highly susceptible to infection with *Leishmania donovani* when the animal is inoculated intraperitoneally with infected spleen pulp.

In one series of 8 or 9 hamsters inoculated intraperitoneally 91 per cent were infected the *Leishmania* being found intracellularly in the animals from one to 33 days after inoculation the spleen becoming infected as early as the third day and the bone marrow in 46 days.

More recent work by Young Hertzog and Lan confirmed the susceptibility of this hamster to infection. However there was a tendency to recovery from the infection as was shown by the fact that out of 837 animals which were positive by liver puncture during life 20 were negative at autopsy. This hamster also became infected after intraperitoneal injection of cultures. However in a series of scarification experiments with parasites from the organs of infected animals only 18 out of 63 became infected. Feeding experiments were also performed in which hamsters ingested countless numbers of parasites but infection was not secured. However Shortt Caghead Smith and Saminath later reported that hamsters can be infected with *Leishmania donovani* by

the oral and conjunctival routes if parasites from the spleen and liver of other hamsters or cultural forms are ingested. They think these experiments prove that the assumption that the parasites whether in the *Leishmania* or *Leptomonas* form are unable to survive in the intestine because of the presence of the bacteria is incorrect. They also believe that the hypothesis of oral infection in kala azar is thus reopened.

Mayer has found that the European hamster *Cricetulus frumentarius* is likewise susceptible to infection.

Blanc and Caminopetros have reported that the small Macedonian marmot or spermophile *Citellus citellus* is very susceptible to kala azar, whether the virus is of human or of canine origin. It was easily kept in captivity and should become a useful laboratory animal.

### EPIDEMIOLOGY AND ENDEMOLOGY

Three factors essential for the propagation of kala azar are the primary source of infection, the transmitting agent, and a susceptible population. More or less association between the sick and the healthy also appears to favor the spread of the disease. Apparently through the fact that the healthy are exposed to the same infectious agent that the sick have been. The agent which transmits the infection is influenced to a certain degree both by the local and climatic conditions. Thus, as we have seen, the disease has sharp geographic limitations to certain tropical and subtropical localities and it has not spread beyond its endemic areas in spite of opportunities afforded by commerce, pilgrimages and emigration. It is not usually, however, a disease of very hot climates, and when it occurs in tropical countries it usually prevails particularly in the cooler seasons either during or after the rains.

In India Rogers noted that the onset of the largest number of cases was in the cooler weather in February and in Assam it has long been recognized that the majority of the cases occur in the cooler periods from November to February. Napier who analyzed the onset of 2000 cases in Calcutta found that in June and July there were very few. Then the curve began to increase and reached its height in January. In the Sudan Archibald and also Henderson found that there seemed to be a greater incidence in the period following the rains between August and December although as he emphasizes in considering the incidence of the disease the long incubation period must be borne in mind. Gabba found that in Italy the greater number of cases occurred in March, April and May. Caronia also observed that in Palermo most of the cases began in winter or spring and Abate found in Catania that there were twice as many cases in March and April as in August and September while Spagnolio and Fouzo also observed that most of the cases seen in Italy began in the early spring. Khoudkin in Samarkand noted that both infantile and dog kala azar occurred in the spring of the year particularly in May.

The influence of the climate and particularly of the humidity upon the incidence of kala azar in India has also been emphasized by McCombie Young, who found that the distribution of the disease in India is related to a high degree of humidity combined with a mean minimum temperature not less than 50 C in the coldest months of the year. He also studied the seasonal incidence of kala azar in Assam but found that it varied in different parts of the province. Owing to the long and uncertain incubation period it was difficult to ascertain the seasons of maximum infection.

Napier also points out that a common factor of the endemic areas in India appears to be a degree of humidity which is indicated by an annual mean of daily mean humidities of at least 60 per cent and for at least 3 months of the year a monthly mean of 8 hours humidity of at least 80 per cent. The highly infected areas have even higher degrees of humidity. He also points out that in practically all the endemic areas the normal annual rainfall is above 50 inches.

The temperature conditions common to the highly infected areas are a monthly mean maximum temperature that is always below 100 F and a monthly mean minimum temperature that is above 45 F, a mean annual diurnal range of less than 20 F and a diurnal range of less than 12 F for at least 3 months of the year. In a few instances in the areas of low endemicity the monthly mean maximum temperature rises to 105 F and the annual mean diurnal range is as much as 24 F.

However in China the influence of these climatic factors has not been observed. In China Young and Hertig found that an analysis of the records concerning about 850 kala azar patients treated at the men's hospital at Hsuehchowfu revealed no evidence of any seasonal variation in the disease as far as could be judged from the time of onset as given by the patient or the date of admission to the hospital. These findings agreed with those of Patton and Hindle based on the study of 301 cases in Shantung. Young and Hertig also point out that the limits drawn by Napier for various climatic factors such as temperature, humidity, rainfall, etc. which correspond with the distribution of kala-azar in India appeared to have little influence on the occurrence of the disease in China. The climate in North China is of course more temperate than that of India and the rainfall is more moderate about 5 inches.

Two outstanding features of the disease both in India and China are that it is generally confined to alluvial plains and does not usually occur above a greater height than 2000 feet above sea level. A notable exception reported is that of Savage who observed 2 cases of kala azar in European boys attending school at Sanawar in the Simla Hills and the few cases found in the Garo Hills.

The disease is also one of rural districts in both India and China. Young and Hertig point out that in China no large city is known to have much kala azar though it may be surrounded by heavily infected villages. Napier and Muir also state that in India it is essentially a disease of rural districts as against towns although it does occur to a lesser extent in towns. They found that it was often rampant in old established villages sheltered by abandoned vegetation whereas newly established and more open villages in the immediate neighborhood were free from the disease.

In districts around Calcutta it has been noted that it is associated with soil unprotected by pavement or cement with abundant vegetation and with ground floor residences also under certain conditions with insanitary surroundings more especially when these were connected with the presence of chickens or ducks. In China chickens are housed or kept in the vicinity of the houses and overcrowding in the houses is very common as is the case in India. However Napier believes that crowding does not influence the prevalence of the disease. In India as in most countries kala azar is limited more or less to low lying districts and to areas adjacent to stagnant or running water. In Italy likewise the disease appears to occur only at low altitudes and to favor country districts rather than towns. When occurring in the latter it is particularly confined to the outskirts (Caronia, Spagnolo, Adler and Theodor). In the endemic areas of Italy it has been noted that the houses are ill ventilated, overcrowded and more often occupied by domestic animals more particularly dogs.

In the Sudan as in India the disease occurs particularly in villages adjacent to lakes and rivers where incidentally malaria is also endemic. Archibald emphasizes that it has never been found in arid areas or desert. In India kala-azar has been



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emphasized as a house and family infection in certain localities but *this is rather the exception in other countries*. In the Sudan it has been exceptional to find more than one case in the same family and in the Mediterranean regions cases of infection in the same family have been noted but are rare.

Young and Hertig point out that the inhabitants in Chinese kala azar areas have little malaria or hookworm infestation although in India and in the Sudan these are common among the kala azar cases. In fact Napier lays great stress upon the fact that in India practically all kala azar endemic areas are malarious though of course the reverse is not true. Turkhud, Krishnan and Seetharam also found that in Madras in every kala azar endemic area which they visited there was a very close correlation between the degree of incidence of kala azar and of malaria. They believe that a malarial attack is a common precursor of kala azar in India and that malaria plays an important part in the epidemiology of the disease by lowering the resistance to infection of the individual in a community.

McCombie Young also considers that epidemics of kala azar in India have been determined by circumstances which have periodically lowered the general vitality of the inhabitants for example the influenza pandemic as well as epidemics of malaria. Napier and Gupta also emphasize that the susceptibility of the population plays an important part in epidemiology. Thus the history of the recent epidemic of the disease in some parts of Assam suggested that there both local and climatic conditions are always suitable for transmission and that the epidemic was brought about by a temporary increase in the suitability of the climate combined with a depression in the resistance of the population possibly produced by the influenza epidemic so that wherever infection was introduced in the form of a case of kala azar epidemic conditions prevailed.

*On the other hand in endemic areas in Bengal such as the one investigated in 1931* when there was a wave of exacerbation of the disease it was more widespread and less catastrophic. Moreover it tended to last longer unless the circle was broken by treatment of the infected persons. Young points out that under normal conditions in these endemic areas chiefly children between the ages of 8 and 10 years are attacked children of other ages less frequently and adults comparatively rarely (and then possibly only when in a low state of health or when they have been rendered susceptible by some specific infection such as malaria). In special circumstances when infection in a village becomes very intense the incidence among adults rises but this does not occur frequently.

These facts suggest that the primary source of infection is always present that the local conditions are suitable for transmission as also are the climatic conditions for some part of the year and that the population always contains a few susceptibles. The balance is upset by a temporary increase in suitability of the climatic factor or by the lowering of the general resistance of the population by some epidemic when there is immediately a general exacerbation of the kala azar throughout the whole area. The factor which limits the extension of the disease is apparently the individual resistance of the majority of the population. While many are exposed to infection few acquire it and still fewer develop the clinical syndrome kala azar.

In the endemic centers in China Young and Hertig also point out that there has been no single gr. at epidemic comparable to that of Assam. However a well marked feature of the disease in China is that it tends to occur in cycles of 15 or 20 years but the incidence in each area or even each village may have no relationship whatever to that of neighboring ones. This cycle is characterized by a mild epidemic extending over 4 or 5 years reaching varying intensities and then declining to a few or no cases for 10 or 15 years. They believe that the decline of these local epidemics cannot be attributed to the treatment and cure of cases as has been suggested by Shortt and

Young in connection with the same phenomena in India since histories of similar epidemics recurring every 15 or 20 years with spontaneous declines are repeatedly related by the older inhabitants of China both in Shantung and Kiangsu.

The discovery of the presence of the condition known as dermal leishmaniasis as a sequel to the generalized infection or visceral disease has been recently noted as an epidemiological factor of importance. Napier and Gupta in their recent study of the epidemiology of the disease in India say that they find evidence to show that the leishmania infection persists in the skin of a number of the persons who have been treated for visceral infection. Their figures indicated that 6 per cent of the treated patients of Calcutta showed clinical signs of the dermal infection and they suggest that in a larger percentage such infections exist subclinically.

Usually about a year but sometimes several years elapse between the cessation of all symptoms of the visceral disease and the first manifestations of the dermal infection. Napier and Gupta believe that patients with these dermal lesions whether accompanied by other clinical symptoms or not may constitute reservoirs of infection. It has been found that sandflies can be infected by feeding on dermal lesions not only on nodules but also depigmented areas. Napier and Gupta believe that eventually with the passing of each epidemic wave both in Bengal and Assam the clinical picture of leishmania infection will gradually undergo a change from the visceral to the dermal manifestations.

The fact that frequently in a household a single case of the disease cropped up year after year led Napier to wonder how the source of infection was maintained in the absence of a clinical case of the disease and the question of an alternative host—mammalian, avian or reptilian—arose. This of course has been investigated not only by Napier and Gupta but also by other workers with entirely negative results. However Napier's observations regarding the wide spread occurrence of leishmanial skin lesions have made the alternative host hypothesis unnecessary. He remarks that the skin lesions are very chronic and might constitute a low grade source of infection for many years. If this theory is correct the incidence of skin infection should be a measure more or less of the kala azar endemicity in any particular area.

However while in Bengal the incidence is high in other areas in India as well as in China and elsewhere there is no evidence that dermal lesions prevail or exist except in a very small percentage of the cases. Also the kala azar patients with visceral lesions are probably a much wider source of infection than those with skin lesions alone.

Napier points out that the population of Bengal has now been subjected to infection with kala azar for some generations consequently when the disease appears there even when no treatment is given epidemic conditions do not usually arise and the dermal lesions are far more common sequelae than they are among the inhabitants of the more recently invaded Assam Valley where epidemics have particularly occurred. In the latter province as the general immunity of the population rises through repeated outbreaks of kala azar it is suggested that dermal lesions may be expected to become more common.

In their theory of the etiology and epidemiology of the disease Napier and Krishnan lay stress first upon the host resistance or immunity and second upon parasitic variability. They point out that there is evidence of the existence of a natural immunity as the disease does not follow the introduction of the parasite in every case. The natural immunity differs in different individuals and children of certain ages are much more susceptible than adults.

There is also evidence of the existence of an acquired immunity since after establishment of the infection spontaneous cure may occur. This acquired immunity in persons who survive the infection is complete and is maintained for a long time since second attacks are almost unknown. They believe it permissible to assume that after generations of subjection to infection the host population in a specific area will undergo a change as regards their immunity.

**Age**—Age has an influence upon the epidemiology of visceral leishmaniasis. Thus in the Mediterranean area the infection is more common in infants from about 1-4 years of age while in China it is more common in older children and in India young adults are most frequently infected. Nevertheless in all these countries the infections may occur either in infancy or adult life. In earlier years kala-azar was thought to be more a disease of adults in India while in more recent years cases in adults have been found to be more numerous in the Mediterranean areas than was formerly supposed.

**Sex**—Statistics in general show that males are more frequently attacked than females. Under nourishment and debility are predisposing causes.

**Occupation and Race**—The disease is rare even in India amongst the better classes of white people American and Europeans. A few cases among such people have been reported in Calcutta in recent years and European planters living in comparatively well kept bungalows on tea gardens are occasionally attacked. However while in general racial religious and cast distribution of the disease in India have been found to be fairly even in his most recent survey Napier (1930) found that the percentage incidence amongst Christians was almost double that amongst Hindus. In 8 out of 9 villages with a purely Hindu population the incidence was very considerably less amongst the general Hindu population. He points out that the environment in which the Christians live is much more suitable to sandflies than that in which Hindus live. He believes the keeping of ducks and fowls in the compounds and even in the living rooms is liable to foster a general unsanitary state of affairs and at the same time would undoubtedly favor the breeding of sandflies.

No occupational prevalence has been observed either in India or in the other endemic centers.

## TRANSMISSION

It has generally been conceded that an infected individual is essential to the cycle of transmission of kala azar and since the discovery of the leptomonas type of *Leishmania* in artificial cultures and the close resemblance of these to the flagellates encountered in different insects it has been assumed that *Leishmania donovani* had an intermediate insect host. For many years the search for such a host has been made and a very large amount of investigation has been carried out upon this subject both with reference to the Indian and Mediterranean kala azar. The parasites are often present in the peripheral blood in cases of kala azar and here they may be readily ingested by certain bloodsucking insects while the flagellate forms which develop in cultures of *Leishmania* evidently represent a developmental phase which also occurs in some insects. The life history of the parasites of human trypanosomiasis in which an invertebrate host had been demonstrated also suggested the transmission of kala azar by an insect.

The bedbug was first suggested by Rogers as a possible carrier of kala azar, especially on account of the house and site nature of the infection and its incidence among the poorer class of Anglo Indians and Indian Christians in whose houses it was always found.

In 1907 Patton showed that if bedbugs, *Cimex rotundatus* were fed on the peripheral blood of certain cases of kala azar the parasites assumed the flagellate form and multiplication took place within the intestinal tract of the bugs. Wenyon (1911) and Patton (1912) showed that a similar development takes place in the intestinal canal of bedbugs which have fed over an unbroken oriental sore or in the neighborhood of such a sore.

A number of investigators have since shown that *Leishmania* are still infective to experimental animals after a sojourn within the bedbug. Blacklock and Lounie (1931) have demonstrated that viable forms of *Leishmania* can be passed up to 35 days in the faeces of artificially infected bedbugs *Cimex lectularius*. The strains of *Leishmania* employed in their experiments were *Leishmania tropica*, *Leishmania donovani*, *Leishmania donovani* var. *infantum* and *Leishmania brasiliensis*.

More recent work has however shown (1) that while the parasite does develop in this insect it does not do so readily. (2) Infection of the salivary glands or mouth parts has not been observed, but the contents of the hind gut have been shown to be infected. (3) The parasite in the stage in which it is present in the hind gut of the bug is capable of causing infection in an animal when artificially injected. (4) Infection of an animal has not been produced through the agency of the bedbug by any means that could conceivably be reproduced in nature. (5) A bedbug naturally infected with *Leishmania* has not been reported.

**Fleas**—In the Mediterranean region the frequent association of human and canine kala azar both considered to be caused by *Leishmania donovani* suggested as a possible vector some insect common to both man and dogs.

Basile (1911) first reported that the dog flea when fed upon spleen juice containing *Leishmania* became infected with cultural forms of the parasite. Later on he announced that he had found flagellate forms of *Leishmania* in the human flea. He also claimed to have transmitted the disease from dog to dog by fleas. However neither Wenyon nor DaSilva in a very careful series of experiments were able to confirm Basile's observations. They obtained no evidence whatever of such a development of *Leishmania donovani* in either the dog or human flea *Ctenocephalus canis* and *Pulex irritans* and came to the conclusion that the flea is not the transmitting agent of kala azar. This was later confirmed by the experiments of Nicolle and Anderson and Napier.

**Sandflies**—Sandflies of the genus *Phlebotomus* were also proposed as being the agent of transmission. These flies were first suggested as possible vectors of *Leishmania tropica* in the Mediterranean area by Pressat (1905) and the Sergents (1905). Wenyon in 1911 suggested them as vectors in Bagdad. Mackie (1914) and Acton (1919) thought them vectors of *Leishmania donovani* in India. However the experiments of Knowles, Napier and Smith (1925) particularly attracted attention to the importance of sandflies in the transmission of kala azar in India. In that country Sinton found that the distribution of *Phlebotomus argentipes* coincided generally with that of kala azar.

Studies carried out upon this fly in Calcutta showed that 25 out of 56 female flies which were bred in the laboratory contracted a *Leishmania* infection after feeding on kala azar cases. Bred flies 46 in number fed on control cases acquired no such infection while 407 wild flies 317 females and 90 males also showed no infection. Similarly 20 wild *Phlebotomus minutus* were uninfected. However it was found that this species of the fly would not feed on man. Also 103 unidentified wild sandflies were found uninfected. These observations were confirmed by Christophers, Shortt and Barraud (1921) who constituted a kala azar commission in India to investigate the mode of transmission of the disease. They found abundant flagellates in the fed flies.

up to the fifth day by which time the flies invariably died. Later by refeeding the flies after 4 days they could sometimes be kept alive to 8 days after feeding and some of these also showed infection. Napier and Smith in 1926 and 1927 carried out experiments during a year in which 2338 sandflies were fed on infected cases of kala azar with a proportion of success varying from 40 per cent infected in March to nothing in December and January few flies being available in the latter months owing to accidental loss. Flagellation was found to be most active in the flies in the hot humid months June to September and slow or absent in the coldest months. Development sometimes occurred in the flies even when no parasites could be found in the circulating blood of the patients upon which they were fed indicating that great multiplication of *Leishmania* has occurred in the fly's gut.

Further investigations showed that in *Phlebotomus* flagellates became abundant in the mid gut by the third day after an infected feed and move forward to the pharynx and mouth cavity on the fourth or fifth day. On the seventh to ninth day after the flies have fed a second time the flagellates often invade the proboscis and it is presumed they must be inoculated into the skin when the insect bites again. This is in striking contrast to what happens with true herpetomonads which naturally inhabit insects and which tend to move backwards toward the rectum and discharge *Leishmania* forms which may be encysted in the faeces.

Shortt, Barraud and Craighead concluded that only experimental transmission by the sandfly would now seem to be necessary to prove finally the role of this insect in the transmission of kala azar. One sandfly was caught in a kala azar house with the remains of a blood meal visible. It was kept for 3 days until it died, when dissection showed a heavy flagellate infection especially in the regions of the proventricular fold but not more anteriorly. This was the first naturally infected fly found.

Young and Hertig working in China found that *Leishmania donovani* in a suspension from hamsters' spleens when injected into the coelomic cavity of sandflies flagellates and multiplies for at least 9 days in *Phlebotomus sergenti* and that such flies are then capable of producing an infection when injected into hamsters. However they were not successful in obtaining infection of hamsters by the bites of sandflies fed on kala azar cases or other infected animals. Similar tests were made by Patton and Hindle.

Adler and Theodor working in Catania with *Phlebotomus papatasi* and *Phlebotomus perniciosus* and their relation to infantile kala azar fed these flies on hamsters infected with *Leishmania donovani*. There was a marked difference however in the percentage of the two flies that became infected. *Phlebotomus perniciosus* gave a high rate of infection but *Phlebotomus papatasi* a considerably lower one from which it is concluded that *Phlebotomus papatasi* can be excluded as an important vector of kala azar in Italy. Further they showed that when the proboscis of a sandfly is inserted into a capillary tube containing a solution of citrate of soda the mouth parts may exhibit all the movements of piercing with or without ingestion of fluid. Employing this technic according to Hertig's method with 15 *Phlebotomus perniciosus* which had ingested *Leishmania* 5 to 13 days before it was found that in 6 instances the fluid in which the proboscis had been inserted contained flagellates which varied in number from one to hundreds. In all cases however the number was small compared with that of the flagellates found in the flies on dissection. From these experiments it appeared evident that the flagellate forms of *Leishmania* were able to leave the proboscis of *Phlebotomus perniciosus* during the act of biting a fact which might explain the relative frequency of Mediterranean kala azar in infants under 12 months of age. They also showed that when ingested by *Phlebotomus papatasi* *Leishmania tropica* whether from the cultures or from the

sore multiplies ascends the cardia and pharynx and in a few cases enters the proboscis. Three strains of *Leishmania donovani* of Mediterranean origin also behaved in a similar manner. Hundle also has obtained results similar to those of Adler and Theodor.

Shortt, Craighead, Smith and Swaminath performed experiments in which an attempt was made to transmit kala azar to 4 volunteers by means of the bites of infected *Phlebotomus argentipes*. These however failed as did experiments upon 22 white mice and 15 Chinese hamsters. In a second experiment with 7 volunteers a larger number of infected flies was employed. The 7 volunteers had never been in a kala azar area and as in the first experiment precautions were taken to exclude other sources of infection. The flies used were exclusively those having their third or subsequent feeds as it seemed unlikely that flies at an earlier stage can be infective for it was found that after the second feed the heavy infections with flagellates of the anterior part of the alimentary tract occur. This second human experiment also resulted negatively although the intensity of feeding by flies was immensely greater than would have been obtained under natural conditions. As a result of the failure to produce human infections as well as infection of mice and hamsters by the bites of *Phlebotomus argentipes* the authors remarked that they can only suppose that some essential factor in the process of infection has been omitted or that a vast amount of labor has been expended during a period of 5 years on an insect which is not an essential link in the chain of infection. F. Knowles suggests that the failure to transmit the disease by infected sandflies may be due to a natural resistance which must be lowered before infection will occur.

However later Shortt, Smith, Swaminath and Krishnan announced that although they had made numerous attempts in the past to bring about the transmission of Indian kala azar by the bite of *Phlebotomus argentipes* they are now able to record the first successful transmission of it to an animal by the bite of this insect. This occurred in a hamster upon which 144 infected flies were fed. Apparently the only detail in which the present experiments differ from all the previous ones is in the somewhat longer period between the commencement of the experiment and its termination by the post mortem examination of the animal. This period was one of about 17 months. They do not think this an important point since the hamster showed no macroscopic enlargement of the spleen—a fact indicating the probability of a comparatively recent infection—that is an infection occurring late in the series of feeding experiments. The fact that only 1 out of 42 hamsters experimented on along similar lines became infected indicates that the infection rate by the bite of *Phlebotomus argentipes* may be a low one.

Napier, Smith and Krishnan (1933) reported additional successful transmissions to hamsters by the bite of *P. argentipes* following repeated feedings and Smith, Lal and Mukerjee (1936) have reported a fourth successful transmission by this animal. It is considered by these investigators in view of these successful transmissions up to date the role of the sandfly in the transmission of kala azar cannot be justifiably excluded and they think that better results would possibly be obtained if the laboratory technique could be modified so as to obtain more natural methods of feeding *P. argentipes*. Smith et al (1940) report more successful infection of hamsters with flies nourished on fructose.

Smith, Halder and Ahmed (1941) have since fed *P. argentipes* upon a patient with kala azar and the insects were then maintained for 10 days



on a diet of raisins. At the end of this time they were offered a blood meal on experimental animals of 5 hamsters and 8 mice, upon which they fed. All the animals except 6 mice became infected.\*

In view of the negative results of most attempts to infect by the inoculative method with this fly it had naturally been suggested that the insect may be instrumental in infecting by other methods such as by interrupted feeding or when being crushed on the skin the parasites entering either the wound of the bite or being rubbed into another lesion. However the contaminative method by which the insect passes infective faeces on the skin the faeces then gaining access to the wound made by the sandfly in biting is considered as less likely to occur because of the failure to find any other stage of the parasite in the posterior portion and on account of the special anterior development of the flagellate.

**Human Susceptibility**—In regard to the failure to transmit to human beings infection through the bites of infected sand flies, it is important to review the susceptibility of human beings to infection. Maggiore (1925) inoculated infants with bone marrow and cultures of *Leishmania infantum* but obtained negative results. He also failed to infect babies by the inoculation of *Leishmania tropica* from cases of oriental sore. On the other hand Adler in Jerusalem, succeeded in infecting 5 out of 6 adults with this parasite by direct inoculation from human lesions. However, Adler and Theodor failed to infect an adult human being by the inoculation of the entire mid gut of 2 sand flies *P. perniciosus*, which had become heavily infected with *L. infantum* after feeding on an infected Chinese hamster. Adler believes that adults possess towards *L. infantum* a relative immunity which, however is not due to previous infection with this species.

Adler thinks the experiments inconclusive in regard to the failure in India to infect volunteers by infected sand flies *P. argentipes*, in that there was no information available regarding the invasion by the flagellates of the epipharynx of the flies employed and it is therefore not known whether any flagellates entered the skin of the volunteers in these experiments.

Human beings are obviously at times peculiarly resistant. Napier (1931) reported that he has on 2 occasions failed to become infected after driving a hypodermic needle into his finger immediately after withdrawing it from the spleen of a kala azar patient. Da Cunha and Chagas (1937) failed to infect 2 human beings by the inoculation of 4 cc. of a rich culture of *L. chagasi*. Finally Adler (1940) has attempted to transmit kala azar to 5 human beings suffering from advanced malignant disease. They were all given massive injections of cultures of *L. donovani* and additional injections of *Leishmania* from the livers and spleens of infected Syrian hamsters and were observed for periods of from 8–17 months. Only one of the individuals who was suffering from cylindrical cell carcinoma of the stomach became infected after the inoculation of the cultures of *L. donovani*. The incubation period was less than 5 months. The patient was observed during 9 months and during this time showed no signs of symptoms of kala azar in spite of the fact that there was a very heavy infection. There was no marked enlargement of the spleen, no fever and no leukopenia.

It may be that animals such as hamsters and even dogs may be more susceptible to infection than man.

Whittingham (1932) has pointed out that since it takes 7 days for the development of *Leishmania* in the sand fly since the average \* the

\* Swaminath Shorff and Anderson (1942) now report 5 human infected by bites of experimentally infected flies.

fly is only 14-16 days and since if it fed on only the first the fourth or fifth and the eighth or ninth day it obviously could only reinfect an individual or animal if it had bitten an infected person during the first feeds

Southwell and Kirshner (1938) after reviewing the question of transmission of the disease have concluded that it is not yet proved that infection results from the inoculation of the flagellate forms of *Leishmania donovani* from the bite of the infected *Phlebotomus* that in the sand fly both leptomonas and leishmanoid forms occur in the mid gut and that transmission of kala azar may be the result of the deposition on the skin of *Leishmania* forms when an infected insect is crushed

Wenyon (1939) however comments that successful infection has been produced by the bites of sand flies in India and that fluid on which infected sand flies have fed and which contains the flagellate and not the *Leishmania* forms may be infective

Other species of *Phlebotomus* than *P. argentipes* as mentioned are believed to be concerned in the transmission of *L. donovani* in other parts of the world Adler and Theodor in Italy and Sicily reported *P. perniciosus* as the most important and in certain Mediterranean areas *P. major* In China Young and Hertig and Chung and others reported *P. chinensis* In Spain *P. papatasi* is thought to be the transmitter in the Sudan and Ethiopia *P. langeroni* and in Brazil and Paraguay *P. lut* & *P. intermedius* and *P. longipalpis* have been found to become infected when fed on infected dogs

**Other Blood sucking Diptera.**—There is no positive evidence that other blood sucking diptera such as lice reduviid bugs ticks mites *Stomoxys tabanids* mosquitoes or *Culicoides* are in any way concerned in the transmission of kala azar Recently Blenc and Caminopetros (1930) have claimed that Mediterranean kala azar may be transmitted by the tick *Rhipicephalus sanguineus* The experiments however merely show that *Leishmania* will survive for about 2 weeks in the tick as they are known to do for longer periods in the bed bug Their claim that the tick will transmit the infection is based merely upon the fact that they were able to produce infection in a spermo-philic *Citellus erillus* by the subcutaneous inoculation of an emulsion of infected ticks which had previously fed upon an infected dog Loyens and Stuet (1939) have suggested that the dog louse *Linognathus setosus* will bite man and might sometimes transmit the infection to man

**Other Routes of Infection**—Other routes of infection in kala azar such as by the alimentary tract respiratory system or by direct contact have also been investigated particularly on account of the failure to obtain more positive results in transmission of the disease through the bites of blood sucking diptera

Young and Hertig and Napier point out that such routes of infection seem generally to be ruled out by the sharp geographical limitation of the disease and its failure to spread into areas outside the endemic regions or above altitudes of about 600 meters in spite of opportunities afforded by commerce pilgrimages and immigration

The idea of infection by the alimentary canal by contaminated food or water has been supported by the fact that in a few instances the para-

sites of kala azar have been demonstrated in the urine, and that they are also present in the intestinal lesions and may even be demonstrable in the faeces. Napier and Gupta think that the parasite probably only occurs in the urine accidentally when either blood or other leishmania infected cellular material occurs there. As dysentery and diarrhoea are not uncommon complications, the escape of the parasite by the intestine seemed more probable.

Christophers (1904) and the writer (1908) found in sections of the intestinal ulcers intracellular *Leishmania*. Perry (1922) reported the discovery of *Leishmania* in large numbers in the jejunum in 2 cases of kala azar. There had been considerable proliferation of endothelial cells in the villi and these cells were filled with parasites.

Meleney however believes from the study of the intestine of hamsters that the parasites are not eliminated from the reticular endothelial system into the intestine. However if ulceration occurs it obviously would be possible for the parasites to escape into the lumen of the gut as does occur in human cases of kala azar.

Shortt more recently demonstrated the parasite in the faeces of human cases. The subject of oral infection has received considerable attention during the past few years and there is some evidence that infection of animals at least may occur in this manner.

In some experiments especially the hamster has been infected by feeding material containing flagellates from the liver and spleen of kala azar cases and hamsters feeding upon other hamsters that have died of the infection became infected. There is however no direct evidence that man contracts the disease in this way.

Khaw who particularly studied the question of oral infection of hamsters suggests that while ingestion as a mode of transmission is negligible in man it may be important in the maintenance of kala azar in the reservoir rodent hosts.

The possibility of droplet infection has been considered. Forkner and Zia (1934) have demonstrated typical *Leishmania* in material obtained by passing a swab over the nasal mucosa. Such smears were made from 15 patients suffering with kala azar and in 9 of these the *Leishmania* were found. Smears from the surface of the tonsil and from the saliva in 1 of the cases showed the presence of *Leishmania*. The tonsil of this patient who died as a result of kala azar and secondary infection, at autopsy was shown to be massively infected with *Leishmania*. *Leishmania* in the nasal discharge of 2 patients was shown by inoculation into susceptible animals (hamsters) to be viable and capable of producing infection.

Shortt and Swaminath (1939) have also reported *Leishmania donovani* in the nasal mucus of 6 of 15 patients in Indian kala azar and Henderson (1939) in 300 patients in the Sudan found *Leishmania* always in the spleen in 1 per cent in the blood and in 7.5 per cent in the nasal mucus.

## PATHOLOGY

At autopsy of cases of kala azar in addition to the emaciation there is frequently marked muscular atrophy, together with oedema enlargement of the spleen and often of the liver. In some cases there are ulcerations or necrosis of the mucous membrane of the intestine and sometimes haemorrhages and lesions of the skin.

**The Spleen**—In advanced cases the capsule of the spleen is usually thickened and perisplenitis may be present. The organ may weigh as much as 3 lbs (1360 gm). In India it is said sometimes to reach 10 lbs.

in weight in the adult. It is usually much congested, firm and deep red in color, unless altered by malarial pigmentation.

The consistency varies somewhat with the stage of the disease. It is generally soft, the pulp being increased. The malpighian bodies are often not visible. In the spleens of more chronic cases the consistency is firm. There is less pulp and in some instances the organ may have undergone more or less fibrous change. Recent hemorrhagic or chronic infarctions may be present. Microscopically the lymph sinuses may be distended, a number of the endothelial cells are swollen and contain numbers of

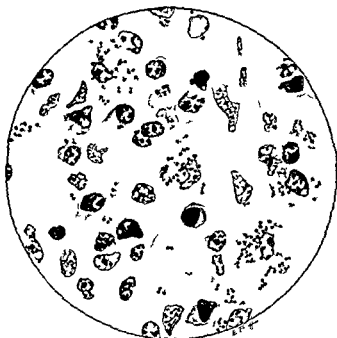


FIG. 59.—Kala-azar Spleen. (From collection of the author's Indian autopsy cases.)

*Leishmania*. Very numerous free mononuclear cells, endothelial cells or macrophages are also observed crowded with parasites.

In the fatal cases studied at necropsy by the writer the diagnosis of the affection was unmistakable from the examination of film preparations from the spleen stained with Giemsa's solution. In such preparations very large numbers of parasites were present, often lying free, isolated or in clumps, or enclosed in large endothelial phagocytes. In the preparation of these film specimens numbers of large swollen cells become broken up and the parasites thus liberated. Usually in sections of the spleen very few extracellular but numerous intracellular parasites are observed (see Fig. 59). The infiltration with large numbers of endo-

thelial phagocytes containing very numerous *Leishmania* constitutes a striking picture. Such phagocytic cells are present both in the reticulum and in the blood sinuses. Numbers of the endothelial cells in the vessel walls are also parasitized. In places the malpighian bodies are infiltrated with such cells. The fibrous tissue may be but slightly increased. However in some very advanced cases the fibrosis is distinct. The pulp is congested with blood, and in addition to the large macrophages there are numerous lymphocytes and plasma cells.

**The Liver**—The liver is usually, but not invariably, enlarged. Evidences of chronic passive congestion or of more advanced fatty degeneration may be present. It has usually a smooth surface, is firm on pressure and on section the consistency may sometimes appear increased due to the existence of a moderate intracellular cirrhosis. Microscopically the liver cells are often atrophied and degenerated. There is usually more or less fatty degeneration. Here as in the spleen, the most striking lesions consist of the presence especially in the lymph sinuses of large numbers of endothelial phagocytes heavily parasitized with *Leishmania*. Some of the pyramidal cells lying along the walls of the venous capillaries (Kupfer's cells) as well as the endothelial cells of the other vessels are also in places swollen and contain the parasites. In advanced cases there is sometimes a marked increase of fibrous tissue with destruction of the parenchymatous cells, but in which parasitized macrophages are sometimes observed. While occasional parasites may be found in the early fibrocellular cirrhotic tissue in the more dense fibrous tissue they are usually not visible.

Shanks and De who recently examined histologically the liver and spleen of 26 cases of kala azar found that only 30 per cent of the livers and only 20 per cent of the spleens showed any increase of fibrous tissue or reticulum. They believe that fibrosis of the spleen and cirrhosis of the liver cannot be considered a regular feature of kala azar itself.

**The mesenteric lymph glands** particularly those in cases in which intestinal ulcers are present often are swollen and contain the parasites. In some cases the *Leishmania* are very numerous in the endothelial phagocytes in the glands and the writer found them in one case as numerous as in the spleen. Napier however says the parasites are often scanty in the lymphatic glands.

**The kidneys** usually show no pathological characteristic changes. Microscopically parasitized macrophages may be seen in the interstitial tissue. The glomeruli are usually normal but the cells of the secreting tubules sometimes show cloudy swelling.

**The Intestines**—Ulceration of the large intestine may occur. In other instances there may be a more general and superficial necrosis of the mucous membrane with no distinct formation of ulcers. Such a necrosis was observed by the writer in 2 cases. Christophers has particularly reported deep and sloughing ulcers. Napier states that he has seldom observed this condition but has seen occasional small superficial ulcers in both the large and small intestines. When extensive ulcerations have been present he has concluded that they were due to some complication in addition to the leishmania infection. Perry found in 2 cases the subepithelial tissues of the wall of the jejunum much swollen owing to the presence of enormous numbers of macrophages packed with *Leishmania*.

Meleney also found in a human case in the jejunum superficial portions of the villi crowded with parasitized cells. There was also found infiltration of parasitized cells

in the ileum caecum, appendix and colon as well as in the jejunum. Meleney believes that the absence of the epithelium in the jejunum in Perry's cases was due to postmortem changes or to the fact that the epithelium was rubbed off during the manipulation of the tissue. In the intestine occasionally the lymph follicles are invaded by parasitized macrophages.

**The Stomach.**—Bauerger has reported finding *Leishmania* in ulcers of the stomach of one case. Meleney also found a few heavily parasitized cells in superficial portions of the stroma of the mucosa of the stomach in one case.

**Bone Marrow.**—The bone marrow also contains numbers of large endothelial phagocytes containing numerous parasites. Occasional myelocytes and polymorphonuclear leucocytes may be seen containing *Leishmania*. The marrow is usually red and soft and contains less fat as is sometimes observed in malaria.

**Other Organs.**—There are no characteristic changes in the other organs. Sometimes endothelial phagocytes containing *Leishmania* are found in small numbers in the interstitial tissues of the suprarenals, thyroid, heart and testes and occasionally in the pancreas, lungs and prostate.

**The Skin.**—In post kala azar dermal leishmaniasis Napier has also found *Leishmania* in the cutaneous nodules. In the study of sections of the skin he found the subpapillary layer oedematous and the fibrous and elastic tissue atrophied. The melanoblasts were well seen. Below this oedematous area was a granulomatous mass consisting largely of proliferating macrophages and fibroblasts. In the center of this mass here and there were multinucleate cells packed with parasites. In a case reported by Shortt the parasites were more abundant in the superficial part of the nodule immediately under the epidermis.

**Summary.**—In the pathological histological changes observed in kala azar it would appear that the parasites after entering the body cause a proliferation of the endothelial phagocytes, many of which ingest the *Leishmania*. In numbers of these cells the parasites apparently multiply within the cytoplasm of the cells and later when such cells rupture the parasites thus set free may be taken up by large mononuclear or polymorphonuclear leucocytes and later appear in the peripheral blood.

When splenic puncture is performed some of the endothelial cells containing the parasites are usually ruptured when both extracellular and intracellular parasites have been obtained in the preparation.

Meleney (1925) has made a careful and extensive study of the histological changes in hamsters which had been experimentally infected with *Leishmania* by Young and Smilie. He emphasizes in comparing the lesions of kala azar infection in the liver with those produced in typhoid infection that in the latter the lesions being stimulated by a relatively toxic organism usually go on to necrosis whereas those of kala azar in which the invading organism is relatively non-toxic are mainly protected in nature and never show necrosis of more than individual cells.

In the spleens of the experimentally infected hamsters he describes islands of clasmatocytes or endothelial phagocytes forming solid masses of tissue in which nearly all of the clasmatocytes contain *Leishmania*. The liver, spleen, lymph nodes and bone marrow were the chief sites of the formation of this tissue. The writer from an examination of the human pathological material of necropses he performed in India has not found such large islands of clasmatocyte tissue in the spleen, liver and lymph nodes. The hamster is a very small animal and in these artificial infections in which very large numbers of *Leishmania* are injected it is not strange that such pictures result as were encountered by Meleney. It seems improbable however that such massive infections occur in human beings.

Sabin and her coworkers by the use of certain colloidal dyes have concluded that the monocytic phagocytes are composed of 2 distinct types of cell the clasmatocytes which arise from specialized endothelium and the monocytes which arise from reticular cells widely distributed in the tissues. The large mononuclears of the blood are regarded as monocytes.

Hu and Cash in the study of experimental hamsters also found that the large phagocytic cells which form the characteristic lesions of kala azar and contain the parasites have the staining qualities of reticulo-endothelium. By marking out the cells of the reticulo-endothelial system of infected animals with intravenous and subcutaneous injections of India ink it was found that the distribution of the parasites and of the lesions is practically limited to this system. In the skin of the infected animals many large cells filled with *Leishmania* were found. These cells took up large quantities of India ink when it was injected subcutaneously and on supravital staining they were apparently clasmatocytes the phagocytic wandering cells of the reticulo-endothelial system. Though the evidence is not entirely conclusive the authors think that it is only in the clasmatocytes the *Leishmania* multiply and that their presence in other cells is more or less accidental. They remark in this connection that the reticulo-endothelial system of Aschoff includes the endothelial system composed of fixed and wandering endothelial phagocytes as well as the reticular system composed of monocytes only. They believe that an investigation of the cells infected with *Leishmania* shows that these are only clasmatocytes and not monocytes except in exceptional instances.

Adler (1940) in a study of a fatal case in which the infection was produced by an injection of cultures of *L. dono an* emphasizes that 2 types of cell infection were noted 1 in which the protoplasm was packed with parasites as in the case of reticular cells of the spleen and Kupffer cells in the liver and 2 in which the cells are relatively slightly parasitized as the adventitial cells of arteries trabeculae of the spleen Glisson's capsules and the connective tissue cells in the stroma of the cylindrical celled carcinoma.

## IMMUNITY

There is not much definite evidence of the mechanism of the immunity in kala azar and no satisfactory method of immunization of man or animals has been discovered. The assumption that the reticulo endothelial system is an important functional unit intimately connected with the process of the immune bodies is particularly based on the fact that the reticulo endothelial cells possess a selective capacity for absorbing foreign particulate matter introduced into the blood stream. It is generally thought that complete blocking of this system by injections of a particular substance is still a problem owing to the well balanced ability of the system to maintain adequately its functional integrity.

Kurotchkin and Chung (1930) have attempted to investigate the immunological response to the administration of bacteria of normal hamsters infected with kala azar and of hamsters treated with colloidal substances. Their experiments have also had some bearing on the question of whether or not the reticulo endothelial cells can be considered as a site of antibody production. Hamsters infected with kala azar were tested for their capacity to produce agglutinins against two species of bacteria *B. typhosus* and *B. proteus* and it was found that kala azar

hamsters responded to immunization with bacterial antigens in a remarkably different way from that observed in normal hamsters. In order to determine the possibility of blocking the reticulo endothelial cells in hamsters by mechanical means different series of these animals were treated with trypan blue and electroferrol before being immunized with bacterial antigens. In this way it was possible to secure considerable suppression of the agglutinin production. They believe their experiments offer substantial evidence that in the case of kala azar the absence of agglutinins against bacteria in injected hamsters is due to the blocking of the reticulo-endothelial system.

Chung and Reimann (1930) have also made attempts to determine antibody formation in kala azar. Nine cases of kala azar, 2 of chronic myelogenous leukaemia and 6 healthy young adults were given 3 doses of triple typhoid vaccine at 5 day intervals. Agglutination tests were performed with the sera before vaccination, after the second vaccination and then at approximately 10 day intervals during 2 months or longer. Though specific agglutinins appeared in all cases they were said to be much weaker in titer and disappeared much sooner in the kala azar and leukaemia cases than in normal controls. Chung and Reimann conclude that their results indicate a relationship between the haematopoietic system which is profoundly affected in this disease and the formation of immune bodies.

The Kala azar Commission in India during 1930 and 1931 have carried on experiments to determine whether mice in which the reticulo endothelial system was blocked were more susceptible to infection with *Leishmania donovani* than normal mice. They found that 1 to 2 cc of a 2 per cent solution of India ink given intravenously in divided doses at 2 day intervals of 1 cc, 0.5 cc and 0.5 cc produced successful blockage. Larger doses killed the mice.

The results of the complement fixation tests in relation to kala azar have been so variable that no conclusions can be reached regarding the presence of immune bodies in the serum.

While the agglutination test may be of doubtful value in differentiating different species of *Leishmania* the recent work of Row (1931) in which killed cultures of *Leishmania donovani* were employed as antigen suggests that the phenomenon of agglutination can be observed with the sera of cases of kala azar. The reaction however is a group one for it occurs also with antigen made from the parasite of oriental sore (*Leishmania tropica*).

Wagener found the injection of alkaline extracts of cultures of *Leishmania* into the skin of rabbits previously rendered sensitive by injections of cultural forms of *Leishmania* produces a local reaction in the form of an erythematous papule which reaches its height in 48 hours and persists from 3 to 6 days. The antigen was prepared from both *Leishmania tropica* and *donovani* but it was not specific for either parasite. Monte negro confirmed this observation also finding that the reaction was not specific in cases of *Leishmania tropica* and *Leishmania brasiliensis*. Ray



prepared various types of antigen from cultures of *Leishmania donovani* and *Leishmania tropica* and through their use obtained clear cut positive skin reactions which he thought were specific in rabbits immunized by vaccines of *Leishmania donovani* and *Leishmania tropica* respectively

Buss has also employed a vaccine made with different strains of *Leishmania donovani tropica* and *bra iliensis*. In 30 definite cases of American leishmaniasis observed in Brazil he found that the intradermal reaction was definitely positive. In 3 doubtful cases the reaction was positive in one and negative in 2 cases. In 10 controls the reaction was negative in 8 and doubtful in 2 cases. For diagnosis, 1 cc of the vaccine was injected subcutaneously. In positive cases a papule was formed with a thick granulation tissue surrounding the site of injection. Later in the course of the reaction giant cells and epithelioid cells were found in this granulation tissue. He thought that pustule formation indicated a high degree of sensitization to the *Leishmania*.

Attempts to treat either kala azar or oriental sore with vaccines have not been carried out extensively. In kala azar the attempts seem to have been unsatisfactory. Killed cultures were used by Longo in 4 cases and by diCristina and Caronia in 7 cases. Jessner and Amster suggested the possibility of employing vaccines for the purpose of immunizing against infections.

Attempts to treat oriental sore by vaccines have given more or less indefinite results (Parrot, Donatien and Lestoquard). Row believed that a vaccine of *Leishmania tropica* was helpful in hastening a cure of oriental sore. Buss attempted to treat with vaccine a few cases of American leishmaniasis but these did not lead to any definite results.

However, there is some information concerning natural immunity or resistance to infection with *oriental sore*. Adler inoculated two people from a sore but in one individual the lesion failed to develop.

Thomson (1931) refers to the fact that an active acquired immunity has been recognized for many years in Mesopotamia where it has been a common practice to inoculate children deliberately on some unexposed part of the body to prevent the disfiguring sores from appearing on the face.

Immunity has been recorded in dogs after cure but it would seem that a previous infection does not always confer complete immunity. According to Marzinowsky and Schourenkoff an experimental sore produces immunity only when allowed to run its full course. Adler found that after the natural cure of an experimental sore on his own arm he completely failed to infect himself subsequently with the same strain and also with another strain biologically different from the first.

In regard to natural immunity in kala azar Napier believes there is evidence that such immunity does exist. He points out that the introduction of a large amount of morbid material does not always produce the disease in animals and that there is considerable evidence of natural immunity in different individuals and points out that children of certain ages are much more susceptible than adults.

It is well recognized that the serum of normal human beings possesses lytic properties for the flagellates of *Leishmania*. Adler found that suspensions of flagellates of *L. donovani* in saline with 10 per cent rabbit serum became destroyed rapidly when 1-10 normal human serum was added. He also found that suspensions of 5 000 000 flagellates per cc in Locke's serum agar became reduced to 2-2 millions in less than 10 minutes through the action of normal human serum. For about a minute the flagellates appear natural. Shortly afterwards the nuclear membrane bursts and the contents of the nucleus is poured into the cytoplasm. This lytic property of the serum was found to be well marked in two cases which he failed to inoculate with *Leishmania* as well as in one case that became heavily infected by an inoculation. In this latter case there was a marked destruction of the parasites in the blood stream at the same time that they were multiplying in the viscera. In view of the fact that he succeeded in infecting only one of the five individuals by direct and massive injections of *L. donovani* he concludes that the factors which determine individual susceptibility for kala azar are quite unknown.

Maggiore (1925) also could not succeed in producing kala azar in human beings inoculated subcutaneously with *Leishmania donovani* and *Leishmania tropica* taken directly from the bone marrow of a case of kala azar.

A study of the epidemiology of Indian, Chinese and Mediterranean kala azar does not give entirely definite information regarding age susceptibility since in India the infants tend to escape infection while under 5 years of age while from 5 to 30 the infection rate is high. In Italy on the other hand the disease is highest in infants and lower in adults. Apparently a similar condition exists in northern China. Adler has suggested that this apparent difference in age susceptibility may be best explained on the assumption that the conditions of transmission in these areas through the agency of sandflies differs. Napier and Gupta have emphasized that kala azar may exist in man in a mild form with few or no clinical symptoms.

Napier and Krishnan believe there is also considerable evidence of the existence of acquired immunity as after establishment of the infection spontaneous cure may occur. Napier points out that while formerly the disease was recognized as a very fatal one it is not always fatal and that some patients recover without any treatment. This acquired immunity in persons who survive the infection he says is complete and is maintained for a long time. After nearly 11 years' experience in one locality during which time he has seen over 10 000 cases of kala azar including many hundreds of cases of relapses he has encountered only 2 cases in which he considers that there was any reasonable evidence of a reinfection having occurred. He thinks there is a possibility that immunity in a person who recovers naturally from the infection may not be of the same nature as that of the patient who recovers after treatment but of the two the naturally acquired immunity is likely to be the more permanent. He also points out that after generations of subjection

to infection the host population in a specific area will gradually undergo a change as regards their immunity

The epidemiological studies of both Indian and Mediterranean kala azar by Young, Paradiso and Napier all indicate that there is a gradual development of immunity established by previous infection. How many mild cases of kala azar exist in endemic areas is not definitely known but it is quite possible that they are much commoner than was previously suspected and may often be overlooked because the infection is mild.

### SYMPTOMATOLOGY

**Incubation Period**—The incubation period has been stated to be usually from 6 weeks to 4 months but it varies greatly. Cases have been reported in which the incubation period was under 10 days (Manson) or only 14 days (Napier and Muir). There is also an instance reported by Napier in which the disease did not develop for 18 months. Manson Bahr points out in a proportion of instances as in some artificially infected dogs the disease like dermal leishmaniasis may remain latent for months.

**Onset**—There is nothing characteristic about the onset which may be either gradual or sudden. Both Napier and Manson Bahr emphasize the difficulty in diagnosing the infection during the first month. When the onset is sudden there is usually high fever which may be preceded by a chill and in some cases by vomiting. The initial fever is frequently severe and may last from 2 to 6 weeks being followed by a period of apyrexia succeeded again by fever and a gradual enlargement of the spleen and liver.

Napier describes a malarial type of onset which he says is the commonest in an endemic area. This form of onset resembles an attack of tertian or quartan malaria the fever rising suddenly to 102° or 103° F accompanied by rigor but not usually followed by sweating and dropping on the following day or the same day to normal, then possibly rising on the third or fourth day and after that becoming somewhat irregular. Since quinine sometimes has a temporary effect on the temperature this type of kala azar fever is seldom diagnosed on clinical grounds alone until later when the quinine resistant nature of the fever becomes definitely established.

In about 20 per cent of the cases in Calcutta the disease is said to have had an onset resembling typhoid fever. The patient becomes ill with a gradually climbing fever which reaches 103° 104° F or higher after a week. A high continuous or high remittent fever continues for 10 days or so and then frequently slowly falls to the neighborhood of 99° or 100° F.

There are seldom any abdominal symptoms but there may be slight diarrhoea. The spleen is sometimes just palpable and there may be a suggestion of tenderness but if the patient has had previous malarial attacks the spleen is likely to be considerably enlarged. The pulse is usually above 100 and the tongue fairly clean. The presence of a double remittent temperature may appear and in the absence of definite abdominal symptoms arouse the suspicion of kala-azar rather than of typhoid fever. Also after a period of almost normal temperature the temperature may again rise and it is some times not until the second relapse with a gradual enlargement of the spleen that the disease clinically suggests particularly kala azar.

In a third type the onset is gradual or insidious and is sometimes so ill defined that even after careful inquiry it may be impossible to date the real beginning of the disease. The patient may have had a feeling of being ill for some months without however any definite symptoms which would cause him to seek medical advice. In some cases

in which there are no striking evidences of the infection the physical examination may reveal an enlarged spleen and spleen puncture show the nature of the infection. Napier states that the rapid type of onset is more frequently observed under epidemic conditions when the disease is established on virgin soil.

**Fever**—The temperature shows great irregularity. Throughout the course of the untreated disease the temperature when recorded on the chart very frequently assumes the form of long irregular waves of fever. The febrile accessions often last from 2 to 6 weeks followed by periods of apyrexia and apparent improvement. Then follow further periods of fever. However during the periods of apparent apyrexia a nightly rise of temperature may escape attention unless the temperature is taken late at night.

Rogers and Napier have called attention to the tendency of the temperature to show variations twice or occasionally 3 times in 24 hours. To the former type of fever the term double remittent or double intermittent

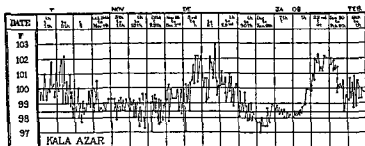


FIG. 6.—T h t f f kala p t d by B tt Smith Th ch rt shows how ea ly one mght onfu th t mp tu arv of th di with t of Malt f v

has been applied. In this type the temperature subsides towards early morning and remains low until about midday. It then rises in the afternoon and subsides again toward evening. At about 8 or 9 o'clock at night it again rises or the second rise may be delayed until midnight when the temperature again subsides towards morning. In order to demonstrate this double rise it may be necessary to take the temperature every three hours day and night. When this type of fever is present it serves to distinguish the disease from typhoid and is practically pathognomonic of kala azar. Napier however states that while it is possibly a sign which is always present at some time or other during the course of the disease in the majority of cases it is often not observed. In the Carmichael Hospital for Tropical Diseases where a 4 hourly temperature chart was kept a definite double diurnal rise of temperature was observed in less than 20 per cent of the kala azar cases during their stay in the hospital. Rogers found that the double remittent type occurred in about one fourth of all cases and in about one half of the early cases.

Sometimes a third diurnal rise of temperature may be recorded and this though said to be equally diagnostic of kala azar is not as frequently

seen as the double rise. A small percentage of the cases may show fever of a high continued type. Napier emphasizes that the patient with a temperature of 102 F in kala azar may be doing his work in the ordinary way and be quite unaware that he has fever. Indeed, while the fever is high and persistent the general symptoms are often slight and there is usually no mental dullness or delirium. Daily rigors are sometimes common early in the disease and sometimes 2 rigors may occur in 24 hours. In other cases they are absent.

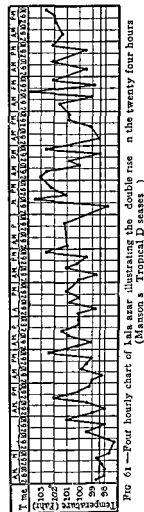


FIG 61.—Four hourly chart of kala azar illustrating the double rise in the twenty-four hours (Manson's Tropical Diseases.)

**General Appearance**—There is usually a progressive loss in weight and when the disease has reached a comparatively advanced stage there is generally great emaciation. The general wasting, the thin arms and face and prominent ribs are usually in marked contrast to the abdomen prominent because of the enlargement of the spleen and liver. In advanced cases there is often much oedema of the legs. The subcutaneous and muscular wasting is also striking. The abdominal veins are often distended. The skin frequently assumes a dusky hue. The conjunctivae reveal little anaemia except in advanced cases. Sometimes marked visible pulsation of the carotids in the neck and rapid pulsation of the heart may be observed through the thin chest wall.

Napier gives as important clinical diagnostic points the marked emaciation, the scanty hair, dark skin, pulsating carotids of the neck, rapid pulse, enlargement of the liver, peculiar soft doughy feeling of the enlarged spleen and the double remittent and quinine resistant nature of the fever. However he points out that there is absolutely no sign nor symptom of the disease which may not be absent upon occasion.

**Spleen**—In the epidemic forms the spleen is almost always enlarged, often reaching several inches below the costal margin. Sometimes it extends to the umbilicus or more rarely even to the anterior superior spine. The

enlargement of the spleen is progressive and usually in less than a month it reaches below the costal margin. In one case of experimental infection (however suffering with carcinoma) the spleen did not become markedly enlarged (Adler 1940).

Napier refers to the fact that the actual size of the spleen is not a very useful diagnostic point and that the teaching which was common in the

medical schools in India to the effect that a spleen above the umbilicus is probably malarial and a spleen below that level is kala azar will certainly not hold today since the disease is recognized in India in a much earlier stage than it used to be. The diminution in the size of the spleen is a useful indication of the progress of the patient under treatment. During intermissions of the fever or attacks of diarrhoea it also sometimes becomes smaller.

Usually the spleen is hard to the touch though in earlier cases of high fever it is often softer. Napier believes that the peculiar soft doughy feeling of a kala azar spleen may be an extremely useful diagnostic point. However he also states that the more chronic the disease the harder the spleen as a general rule. In children at least tenderness of the spleen is common particularly in the Mediterranean areas. In India Napier states tenderness is uncommon and is not complained of in more than about 5 per cent of the cases. He refers to the fact that pain in the spleen may come on suddenly and last for a few days being at first general but soon becoming localized in one spot. This pain he believes may be caused by blocking of one of the splenic arterioles through the invasion of the endothelial cells with *Leishmania* and the formation of an infarct. In the advanced stages of the disease ascites sometimes occurs and when this condition is present it is sometimes difficult to palpate and aspirate the spleen which slips away from the needle more easily.

**Liver**—The liver is usually enlarged after the end of the first month. After 6 months the enlargement is distinct in most cases. Napier found in 300 cases in Calcutta that the liver was palpable in 88 per cent measurably enlarged in 64 per cent and enlarged more than 3 inches in 20 per cent of the cases. He believes that an enlarged thinned out soft liver overlapping a large soft spleen is a condition which is very characteristic of the disease. While a certain degree of tenderness is sometimes present the tenderness is usually insufficient to suggest liver abscess. In Calcutta in a series of 140 patients not suffering from kala azar and for the most part cases of chronic malaria 86 per cent had palpable livers.



FIG. 6.—Kala azar by hanging the patient's abdomen and measurement (C of D S Yangurt, J. Shanghai Sci. Inst.).

**Digestive System and Alimentary Tract** —Stomatitis is not uncommon during the course of the disease. Cancrum oris has been emphasized both by Rogers and Napier as being a very common complication. Cleanliness of the tongue is said to be one of the striking features of kala azar in India, and the appetite is usually good even during the periods of fever. However, the patients do not generally consume a large amount of food. The digestion is sometimes poor and nausea and vomiting occasionally occur.

The Indian Kala Azar Commission have found that a large percentage of cases both of kala azar and of malaria show varying degrees of decreased acidity in the gastric secretion up to complete achlorhydria.

Diarrhoea often appears after the disease has lasted for some time and in the advanced cases dysentery apparently due to severe infection of the intestine by *Leishmania* has been observed. Rogers reported in some of the epidemics dysentery of severe form occurred in 70 per cent of the cases. Occasionally the dysentery has been due to secondary infection either with *Bacillus dysenteriae* or *Endamoeba histolytica*. Napier states that the specific nature of the dysentery in kala azar has never been proved, however a few cases of kala azar have been observed in which dysentery was present during life, and in which the lesions found were not produced by either *Bacillus dysenteriae* or *Endamoeba histolytica*. The work of Christophers and Stratham has demonstrated the specific nature of intestinal lesions due to *Leishmania*.

**Skin** —Certain changes perhaps of a trophic nature, occur in the skin. The characteristic blackening of the skin from which the disease derives its name has been suggested as being due to a certain extent to increased activity of the melanoblasts and also to an intensification of a natural pigmentation due to dryness of the skin. It is most marked over the forehead and temples and occasionally around the mouth, the blackening being intensified by contrast with the anaemic pallor of the rest of the face. Napier has not observed this pseudopigmentation in pure blooded Europeans but states it is very marked in dark skinned Anglo Indians. Manson Bahr says that the dusky pigmentation of the skin is best seen on the feet, hands and abdomen in Europeans but is difficult to distinguish in dark skinned natives. A temporary jaundice is not uncommon. During the course of the disease the skin is apt to become dry, rough, and harsh and the hair is apt to fall out. Septic infection and a tendency to bed sores are very common. Purpuric haemorrhages which usually indicate an unfavorable prognosis are often seen in the skin and are most common on the legs though they not infrequently occur on the trunk. The appearance of purpuric spots may coincide with sudden diminution in the size of the spleen and with other symptoms which suggest the possibility of protein poisoning due to rapid plasmolysis especially in cases under treatment with antimony compounds. The *Leishmania* are sometimes found in the skin particularly in dermal leishmaniasis which is discussed further on p. 270.

**Genito urinary System**—In advanced cases oedema of the legs which sometimes occurs together with puffiness of the face and occasionally ascites as well as diminished output of urine may suggest renal insufficiency. However albuminuria and nephritis are rarely associated with the disease and very few cases of acute nephritis have been reported in connection with it though Knowles reported one case in 1920. During the period of fever in kala azar Napier found that about 30 per cent of the cases showed a trace of albumin in the urine that there was usually not more than a trace and there were seldom casts present. Urobilin is present in a large percentage of the cases. Knowles considers that the presence of urobilin associated with a trace of albumin in the urine constitutes a useful diagnostic point. The chlorides are usually present in normal quantities. Pilcul Ziemann and Waegner and Strothers have reported cases with renal symptoms haemoglobinuria albumin and casts appearing in one case after intravenous injection of antimosan and in the other after stibenyl.

Amenorrhoea is often an early symptom and is almost invariable in well established cases. Napier reports that he has observed a number of cases in which conception occurred early in the disease and in which an uncomplicated pregnancy was continued to full term and resulted in the birth of a comparatively healthy child.

**Nervous System**—Headache may be present but is seldom severe. Even when the fever is high the general symptoms are usually slight and there is usually no mental dulness or delirium. Herpes zoster sometimes occurs particularly when the patient is undergoing treatment with antimony. The condition usually clears upon the discontinuation of the antimony. Retinal haemorrhages have sometime occurred as a complication.

**Respiratory System**—The lungs are normal in the great majority of cases but catarrhal bronchitis may be present in the advanced stages and a terminal pneumonia may develop. In the early stages and also throughout the course of the disease an irritating cough may be present without any marked physical signs in the lungs to account for it. Sometimes this is the most distressing symptom of the disease seriously interfering with the patient's rest at night. It has been suggested that it is probably due to irritation of the vagus from pressure caused by the enlarged spleen.

**Circulatory System**—Haemic murmurs of the heart are frequently present and dilatation is not uncommon. In advanced anaemic cases systolic murmurs are often present. Marked pulsation of the carotid vessels of the neck is frequently visible. The pulse is commonly over 100 and not infrequently 120 even in the early stages of the disease. The blood pressure is generally low the systolic reading being below 100. Oedema of the legs is comparatively common. Napier found this condition present at the time of the examination in 16 per cent of 300 hospital cases in Calcutta and a very much higher percentage gave a history of having



had swelling of the feet at one time or another. Haemorrhages may occur from any part of the body. Epistaxis and bleeding from the gums commonly take place while haemorrhages from the nose, meninges and intestines are sometimes fatal. Purpuric spots and patches are not uncommon particularly in cases in which the prognosis is grave.

**Blood**—Anaemia is a marked feature only in the later stages of the disease though there is always some degree of anaemia after the first month and this is progressive in character. However the count of red cells rarely falls below 2 500 000 in less than 6 months from the beginning of the fever. Napier has found that nucleated red cells are often present as well as cells containing nuclear fragments. Polychromatic staining of the red cells may also occur. In contradistinction to the condition of the blood in malaria the haemoglobin falls in proportion to the red blood cells. The anaemia is of the pernicious type and not of the chlorotic type seen in ankylostomiasis.

One of the most remarkable changes in the blood is the marked reduction in the number and proportions of leucocytes. There are rarely over 3 000 leucocytes found. In one half of the cases which have persisted for 1 month there are often less than 2 000 leucocytes. Napier points out that a leucocytosis does not necessarily exclude the possibility of the case being kala azar as in the presence of any septic complication the white blood count may be markedly increased and even in the absence of any obvious complication it is occasionally above 10 000 per cubic millimeter.

Another important change in the blood is the reduction of white corpuscles in relative number to the red ones. The normal rate of 1 750 or 1 666 red cells is rarely observed. In kala azar the rate is often 1 1 000 or 1 1 500 red cells. Rogers reported in his uncomplicated cases in 90 per cent the rate was less than 1 1,500. This is an important feature in diagnosis and the condition is usually different from that observed in malarial infection. There is also in kala azar a reduction of the polymorphonuclear leucocytes and an increased number of large mononuclears and lymphocytes which may differentiate the disease from typhoid though not from malaria.

When the *Leishmania* are found in the peripheral blood they occur in the leucocytes and it was formerly suggested that perhaps the decrease in the polymorphonuclear cells was due to a destruction of them by the parasites. In children the polymorphonuclear cells may not amount to over 5 per cent of the total leucocytes. In adults they usually do not amount to over 30 per cent if the case is uncomplicated. With the low leucocyte counts the prognosis is unfavorable and terminal infections are frequent. The lymphocytes are increased with the large mononuclears in proportion to the reduction of the polymorphonuclear leucocytes while the eosinophils are decreased. Agranulocytosis has been reported as a complication in a few cases. It is exceedingly rare in India (Das Gupta 1943, Napier 1945).

**Wassermann Reaction.**—The Wassermann reaction has been examined in kala azar particularly by Sutherland and Mitra. In 38 cases 10 gave a positive and 28 a negative Wassermann reaction but in only 2 was the reaction more than slightly positive.

Mu and Hute examined 41 sera from 37 cases of kala-azar in North China by the Kahn test in parallel with Kolmer's Wassermann reaction. The cases varied in duration from 1 month to 4 years. Of the 37 cases only 3 gave a positive Kahn test and in these the Wassermann test gave a similar result. In 2 of the 3 cases there was evidence of syphilis while in the third the test was negative when repeated. It thus appeared that the specificity of the Kahn test was not affected by the high globulin content of the serum which is characteristic of kala-azar. High fever when it occurred likewise did not alter the reaction.

Lloyd Napier and Mitra (1930) examined the blood of 474 cases of kala-azar in which the diagnosis was definitely established by demonstration of the *Leishmania*. They concluded that kala-azar does not give rise to a positive Wassermann reaction.

**Biochemical and Other Changes**—Marked biochemical changes occur in the blood. Rogers and Shorten first reported that the alkalinity of the blood was decreased. More recent work performed by Napier shows that the hydrogen ion concentration of the blood in a patient who is not in *extremis* is practically unaltered. He found that while the hydrogen ion concentration of the blood is about normal its stability was decreased. The coagulability of the blood is frequently reduced and the blood often takes longer than 5 minutes to coagulate in Wright's tubes as was pointed out by Rogers and Napier. Knowles found that the coagulation time might vary from  $2\frac{1}{2}$  to  $5\frac{1}{4}$  minutes with a mean at  $3.28 \pm 0.292$  minutes.

The practical importance of these observations demonstrating delayed coagulation of the blood has been suggested as a contraindication to splenic puncture on account of the danger from haemorrhage. Napier states that in actual practice he has not found this to be an important factor. He also found that the calcium content of the blood was reduced in every case of kala-azar that was tested at the Calcutta School of Tropical Medicine. The normal content for an Indian appears to be from 10.2 to 10.4 mg. per 100 c.c. of serum. In more than half the kala-azar cases tested the content was reduced below 9 mg. and in a few instances it was as low as 8 mg.

Napier also found that a reduction in the blood sugar was a constant finding in this disease. The normal blood sugar for an Indian is 0.1 per cent. In kala-azar it is always below this figure and occasionally it is as low as 0.05 per cent. He also tested the tolerance in a number of cases and found it to be reduced. In most cases a rapid improvement in the tolerance was observed when the patient was given antimony injections. Grieg and Kundu have also found that the blood sugar in kala-azar is usually low.

Sia found that the globulin content of the blood is increased in kala-azar and Ray has shown that there is a very marked increase in the euglobulin element. Lloyd and Paul point out that the euglobulin content of normal serum is on the average 0.16 gm. per 100 cc. or 5 per cent (approximately) of the total globulin. The euglobulin in the serum in well-established cases of kala-azar has been found to average from 1.5 to 2.5 gm. per 100 cc. or from 40 to 50 per cent of the total globulin. These changes in euglobulin and pseudoglobulin with characteristic curves occurred in every case of kala-azar under treatment that had been studied and appear to indicate some deep-seated change in the organism. Lloyd and Paul believe that a special euglobulin appears to be present in kala-azar. This substance or substances precipitated with it are the fundamental cause of the formoleukogel reaction in kala-azar.

More recently Lang has found that the euglobulin in kala-azar is increased 3 to 13 times the normal and 30 to 63 per cent of the total serum globulin. There is also a considerable increase of total serum globulin in kala-azar and an absolute decrease of serum albumin so that the globulin albumin ratio which is normally 0.49 is much increased sometimes to over 4.

The question of the occurrence of agglutinins in the blood serum of kala-azar patients has been made the subject of much study. The antigen used for the tests has generally

consisted of living cultures of *Leishmania donovani* though in a few instances a saline extract of kala-azar splenic pulp has been employed. Several observers have reported positive agglutination in a few instances. However the majority have obtained only negative or inconstant results.

More recently Row (1931) has emphasized the important fact that the use of suspensions of living flagellates in culture is not satisfactory because although a young surface growth of *Leishmania* made up in saline solution with a certain amount of agitation is found to consist of individual and discrete flagellates which actively move about in a hanging drop preparation these flagellates soon become immobilized in masses at room temperature and tend to agglutinate in a few minutes when mixed with neutral control sera even in 1:30 dilutions with normal saline solution. Row thinks this defect can be obviated by killing the cultures of flagellates at 55°C for 1 hour or by exposing them to chloroform vapor. With such an antigen he believes he has demonstrated the presence of agglutinins in cases which were diagnosed as kala-azar both clinically and by the serum globulin aldehyde test. No report was made of *Leishmania* being demonstrated in these cases. He does not claim that this agglutination test will replace for practical purposes clinical diagnostic tests such as the aldehyde or serum globulin reactions or urea stibamine reaction. He also found that the agglutination test is of no value in arriving at a differential diagnosis of the several varieties of *Leishmania*.

The evidence of the presence of immune bodies in kala-azar serum by use of the complement fixation test has been inconclusive on account of the variable results obtained. In these experiments performed by many investigators extracts of kala-azar spleen have usually been employed as antigen. Anderson and Disder (1938) who examined again the complement fixation test in the diagnosis of 9 cases of kala-azar found that it was not sufficiently specific to be of value.

Chung and Reimann (1930) have found that the clinical impression of diminished resistance to secondary bacterial invasion during kala-azar is corroborated by their experimental laboratory evidence of a depression of the immune response to injected bacterial antigens. They found for example a marked depression of the immune response to typhoid vaccinations in patients with kala-azar. After recovery from kala-azar however agglutinins were again formed normally.

Yang and Chen (1930) have studied the blood platelets in kala-azar particularly on account of the haemorrhagic tendency of the disease. Direct counts of the platelets were made by a modification of Thomson's technique. It was found that kala-azar is associated with a thrombocytopenia in which the platelets varied from 50,000 to 100,000 per cubic millimeter. Bleeding from the mucous membranes usually occurred when the lower level was reached. Intercurrent infections might bring about either a thrombocytosis or a further depression of the number of platelets. The patients with a marked thrombocytopenia usually had a severe anaemia and a very low leucocyte count. The bleeding time was prolonged and epistaxis and purpuric rash were frequently observed in such cases. Treatment with urea stibamine caused a further decrease in the number of platelets and this was maintained as long as treatment lasted. During recovery the platelets increased in number.

In a study of 720 cases Keefe, Shaw and Yang showed that all elements of the blood are reduced in kala-azar. However the leucocytes and platelets are decreased before the haemoglobin and red blood corpuscles.

The Indian kala-azar Commission reported in 1931 upon the effect of subcutaneous injection of adrenalin (1 cc. of 1:1,000 solution) on the blood picture in kala-azar. Four untreated cases and 4 under treatment with urea stibamine were used. All the treated cases responded to the injection by a great increase in the number of leucocytes especially the polymorphonuclears and mononuclears (50 to 100 per cent). In the untreated cases the leucocytic response varied in one the increase was as marked as in the treated cases in one it was poor and in 2 there was no increase at all.

Tobias (1942) has reported upon two severe cases of agranulocytosis. In spite of treatment with pentide blood transfusion and adrenalin in both cases death followed a sudden collapse.

**Dermal Leishmanoid**—A cutaneous form of leishmaniasis in which the parasites occur in nodules of the skin has already been referred to in

this article under the discussion of the epidemiology of the disease. This lesion was apparently first reported by Thomson and Balfour in the Sudan in 1909. Brahmachari first called attention to it in India in 1922. In 1927 Acton and Napier reported upon 44 cases of this same condition under the term post kala azar dermal leishmaniasis while in 1930 Napier and Gupta have recorded the study of 150 additional cases. More than half the patients who exhibited the eruption had suffered from kala azar a year or so previously and had received antimony treatment for it.

The condition has been found to occur in all classes of the community and in persons of all ages and of both sexes. Not all of the patients gave a history of having suffered from kala azar and some denied having had any illness which might have been this disease. Nevertheless Napier and Gupta conclude that the condition is usually a sequela of generalized *Leishmania* infection. The dermal lesions usually make their appearance from 1 to 2 years after all signs of the visceral infection have disappeared. In no cases have the dermal lesions developed during the primary visceral attack but in 3 instances there was a relapse of the visceral disease at the time when the dermal lesions were present.

The skin eruption appears as depigmented areas varying in size from pin point to larger patches usually not over 1 cm. in diameter but sometimes coalescing and forming patches occupying the whole of one aspect of a limb. At first the lesions are macular in type but later become very slightly raised. An erythema or a butterfly rash also may occur which varies in intensity in different individuals. The distribution of this rash is very constant namely on the cheeks the skin surfaces of the upper and lower lips and the outer surfaces of the alae nasi. Occasionally it extends to the tip and to the sides of the nose and the chin. Panja (1938) has observed nodular lesions on the tongue in which *Leishmania* were found and notes that this appears to be the first report of such lesions occurring in dermal leishmaniasis following kala azar.

The lesions on the face but rarely on the body may pass on to the nodular stage which is generally reached about a year later.

The nodules are soft granulomatous growths yellowish pink in color varying in size but usually about the size of a split pea. They may join and form plaques. The skin over the nodules is thin and glossy and shows no special susceptibility to break down and heals rapidly after a portion of it has been removed for diagnostic purposes. The nodules are painless but there is no anaesthesia. Rarely they may appear on all parts of the body as well as on the face. While these are the most common types of lesion verrucose papillomatous or xanthoma like types of eruption are described. In other cases there is a hypertrophy of the skin of the lips eyelids and alae nasi.

In the verrucose type warty growths occur at the roots of the nails and on the fingers and toes there is considerable thickening of the distal phalanges of the digits. In the papillomatous type instead of the ordinary nodular growth there is hypertrophy of the individual papillae of the skin with the production of a rough dry area consisting of minute papillomatous growths. These are usually seen on the nose or chin.

In the hypertrophic type the lips eyelids or alae nasi become hypertrophied as if there were lymphatic obstructions of the parts sometimes forming soft lipoma like swellings.

The xanthoma stage would appear to be the final outcome of the condition but it is rarely seen and then generally in cases giving a history of 10 to 30 years duration. The lesions resemble those of xanthoma tuberosum multiplex there being raised orange

colored plaques on different parts of the body most noticeably at the bend of the elbow on the axillary folds on the inner side of the thigh at the outer canthus of the eye on the chin and at the angles of the mouth

Napier and Gupta say that it is not easy to find the parasite in the deep pigmented lesions by direct microscopical examination but by snipping out a piece of skin dropping it into a culture tube of NNN medium the parasite can easily be demonstrated by cultivation On the other hand direct examination of a smear from the nodular lesions usually reveals the *Leishmania* *Leishmania* were demonstrated in 81 cases of the series but in 69 cases only a clinical diagnosis was made

While this condition appears to be prevalent in India in the region of Calcutta apparently very few cases have been reported from Assam and Madras

### COMPLICATIONS AND SEQUELAE

Apparently owing to the changes in the blood in kala azar resulting in a lowering of the general resistance secondary bacterial invasion of the tissues is not uncommon Rogers has emphasized that these secondary bacterial infections produced fatal terminations in the great majority of cases owing to defective phagocytic powers of the blood depending upon the extraordinary leukopenia with great disproportionate reduction in the active polymorphonuclear leucocytes Thus cancrum oris sometimes complicates the disease and its frequency has been emphasized by both Rogers and Napier Perforation may occur and the nose may also become affected These cases are frequently fatal When there is no leucocytosis the prognosis is particularly grave Among the rarer complications may be observed noma of the cervix uteri reported by Rogers Rogers placed the incidence of cancrum oris at 17 per cent However Napier points out that it is not now as frequently seen in India as it was before a satisfactory form of treatment was discovered In Napier's Calcutta series of 300 cases less than 2 per cent were affected

Mastoid abscess otitis media sloughing of the scrotum and ulceration accompanied by herpes zoster have also been observed These complications are common in children in whom the polymorphonuclear leucocytes are often lower than in adults

Lobar pneumonia is the next most fatal complication Bronchial pneumonia also not infrequently occurs

Oedema of the glottis is a comparatively rare complication Three cases have been reported by Napier which proved rapidly fatal He points out that these cases were otherwise progressing very satisfactorily

Dysentery of a specific nature and due to severe infection of the intestine with *Leishmania* is sometimes observed Occasionally the dysentery is due to secondary infection with strains of *Bacillus dysenteriae* or to *Endamoeba histolytica* Rogers reported that in some epidemics dysentery of severe form occurred in 70 per cent of the cases Napier states that dysentery makes its appearance at some stage of the disease with such unfailling regularity that it has frequently been suggested that it is an essential part of the disease However he believes that a secondary causative organism can usually be found to account for the dysentery attacks

Haemorrhages from the nose meninges and intestine are frequent and sometimes fatal Haemorrhage into the peritoneal cavity has been reported by Chatterjee in a

case complicated by ascites. Purpuric haemorrhages are seen in the skin and are most common on the legs but are not infrequently observed on the trunk. When these haemorrhages occur the prognosis is usually very unfavorable.

Ophthalmic changes have been described in several cases by Trantas and Ling.

Oedema of the feet is reported by Napier as a very common complication and general anasarca is occasionally present. However the presence of albumin in the urine is not associated with these conditions nor are there usually any signs of heart failure. He has found that ankylostomiasis will account for a large percentage of them. In parts of China Yates has pointed out that kala azar is almost universally aggravated by ankylostomiasis.

A few cases of acute nephritis have been described but they were probably of an accidental association. Napier has had no experience with nephritis as a complication. A definite progressive ascites is seen occasionally in advanced cases probably due to changes in the liver.

Chronic splenomegaly and hypertrophic cirrhosis of the liver may occur as sequelae of the disease though Shanks (1931) thinks cirrhosis of the liver is unusual. Napier has observed that a large percentage of patients who have undergone treatment with antimony preparations have a typical attack of catarrhal jaundice within three months of the conclusion of the course of injections.

Post kala azar dermal leishmaniasis has already been discussed. Although the majority of the patients with this complication give a history of having had an attack of kala azar there are a few instances in which the patient gives no history of having had the disease and has not received treatment for it at any time.

Malaria and Malta fever have each been reported as complicating kala azar. In India particularly the association of kala azar with malaria is common and important. Ayer, Vasile and Constantino have each reported cases of combined infantile kala azar and malarial infection. Timpano has reported a case of kala azar in a child complicated by the development of acute anterior poliomyelitis.

## DIAGNOSIS

**Clinical Differentiation**—Special clinical features of the disease of assistance in diagnosis have already been mentioned. An analysis of 60 cases of kala azar showed that in order of frequency the following were the most usual signs for diagnosis: enlargement of the spleen, fever, loss of strength, bleeding from the nose, bleeding from the gums, emaciation, anorexia, cough, diarrhoea or dysentery, sweats, chills, abdominal discomfort, amenorrhoea, pigment spots, cancrum oris, and gastric disturbances. In a study of 300 cases of kala azar Napier found that 12.5 per cent began with a double daily rise of temperature and 88 per cent of cases with a double daily rise of temperature proved to be kala azar. Cases with a history of illness of over 18 months were mostly kala azar and a family history was met with twice as often in kala azar as in other cases with an enlarged spleen. Emaciation was three times as common in kala azar especially in the early stages when there was a good appetite. Extreme anaemia was less frequent in kala azar than in other spleen cases. Dilatation of the abdominal veins was twice as common in kala azar. Soft enlargement of the spleen extending 3 to 6 inches below the ribs was more frequent in kala azar while hard spleens extending to 8 inches or more were more common in other diseases. He concludes that probably 80 per cent of the non kala azar cases with enlarged spleen were cases of recurrent malaria or malarial cachexia. Clinically the correct diagnosis of kala azar was made in 88.24 per cent before spleen puncture was performed. This

is said to be a point of practical importance in India where laboratory facilities are so rarely available for diagnosis

Rogers, in commenting upon these clinical observations states that they agree very closely with his own based on several years work in India. Struthers in a clinical study of the disease in China says that in Shantung nearly everyone with an enlarged spleen considers it to be the result of kala azar and comes for treatment. Among such patients he has found cases of myeloid leukaemia, chronic endocarditis, malaria, syphilis and splenic anaemia.

With reference to the presence of fever in diagnosis Napier has pointed out that some cases may be afebrile and Yates (1931) has again emphasized that no fever may be present at the time of examination.

Napier who has had a very wide clinical experience with the disease in India says that a typical kala azar patient has a certain characteristic appearance which it is almost impossible to mistake when one is familiar with it and that an experienced clinician will make a correct diagnosis in 9 out of 10 cases. He concludes nevertheless that when other aid is available it is not right to run the risk of confusing the disease with other infections and that diagnosis on clinical grounds alone is seldom if ever justifiable. It is important to make an absolutely definite diagnosis before treatment is commenced since after treatment has been commenced the difficulties of diagnosis are considerably increased. Napier also emphasizes the injustice of submitting a patient with another disease to a long tedious and unpleasant course of treatment with antimony.

**Differential Diagnosis**—Reference has been made to the fact that cases of kala azar have been frequently diagnosed and treated as malaria. When the case has not yielded to quinine in reasonable time malaria may usually be excluded.

The differential leucocyte count in kala azar often does not help to distinguish the case from malaria but the relation of the red corpuscles to the leucocytes frequently does. The very low leukopenia may be almost diagnostic but leukopenia is frequently present in malaria. In India an extreme general anaemia is more common in malaria than in kala azar. The finding of the causative organism of either kala azar or malaria is sometimes the only definite means of making a diagnosis but it should be remembered that malaria infection may be associated with kala azar.

The disease is also to be differentiated from undulant fever and typhoid fever. Both the serum reactions and the blood examination with the cultivation of either *Bacillus typhosus* or *Brucella melitensis* from the blood or spleen should give aid in the differentiation of kala azar from these diseases. In undulant fever also there is no leukopenia.

Primary tuberculosis of the spleen as well as tuberculosis in association with enlargement of the spleen in any form in which physical signs in the chest are not obvious may give rise to considerable difficulty in diagnosis. In some instances unless the tubercle bacillus is discovered in the sputum or *Leishmania* are demonstrated in the blood or splenic material the diagnosis may be very difficult. Napier thinks that in some cases the positive aldehyde reaction is of much value in diagnosing kala azar.

A syphilitic splenomegaly may also cause confusion in the diagnosis of kala azar. Clinically syphilitic splenomegaly may manifest itself in the secondary stage in the form of a more or less considerable enlargement of the organ often with haemolytic icterus while in the tertiary stage it may assume variable clinical forms and simulate

**Banti's disease** The Wassermann reaction may be of assistance in the differentiation of the affection but it should be recalled that syphilis may be associated with kala azar.

The splenic anaemias particularly Banti's disease and the form of splenomegaly so common in tropical countries to which the name of tropical splenomegaly has been applied may also be confused with kala azar. Tropical splenomegaly of obscure etiology is common in regions in which kala azar is found as China, India and North Africa. As in Banti's disease the syndrome of the affection consists of splenic enlargement with later hepatic cirrhosis and ascites. It also frequently begins at an early age. In the later stages of the disease the blood picture may be similar to that seen in kala azar and indeed kala azar can sometimes only be distinguished from it by the finding of *Leishmania donovani*. Kala azar can usually be distinguished from leukaemia by the blood count.

In Africa Egyptian splenomegaly in which there is often an associated enlargement of the liver and in China schistosomiasis may be sometimes confused with kala azar. Demonstration of the ova of *Schistosoma haematobium* and of *Schistosoma japonicum* in the urine or faeces will obviously be of assistance in differentiating these affections from kala azar.

The absence of hookworm ova and of eosinophilia will aid in distinguishing the disease from ankylostomiasis. However kala azar may be associated with severe ankylostoma infection. In some localities in China Yates (1931) says that kala azar is almost universally aggravated by ankylostomiasis.

Muir calls attention to the possibility of mistaking post kala azar dermal leishmaniasis for leprosy. This is particularly true of two types of leishmaniasis namely that with deep pigmented skin areas and the nodular or xanthoma type. The history of previous kala azar and the discovery of *Leishmania* in the lesion should prevent error in diagnosis.

**Laboratory Diagnosis**—The only conclusive means of arriving at a diagnosis is by the discovery of the parasite *Leishmania donovani*. For this purpose microscopical preparations from the blood, spleen, bone marrow or liver should be immediately hardened in absolute methyl alcohol and stained by Giemsa's or Leishman's methods.

**Splenic puncture** is usually the most valuable method of obtaining material for diagnosis. However the operation is not entirely free from danger as in some cases especially when the spleen is soft and enlarged the puncture wound has continued to bleed and death has resulted. It has been stated that the mortality may approximate 1 per cent in splenic puncture.

Giraud and Gaubert (1938) point out the dangers of spleen puncture and report 3 deaths in 300 cases they performed but point out the risk of spleen puncture is more advisable than submitting the patient to the long antimony treatment in which the diagnosis has only been presumptuous.

Bousefield and Napier report that they have performed the operation more than 2000 times without any ill effects except temporary pain over the splenic area in a few instances. In cases in which the spleen is exceedingly soft it is as well to avoid the operation, one would obviously not puncture the spleen of a haemophiliac and it must not be undertaken until leukaemia has been excluded.

It is recommended that a dose of 30 gr. of calcium lactate in 2 oz. of water be administered the evening before and a half hour before on the morning of the puncture in order to promote the coagulability of the



blood After the puncture is performed the patient should be required to recline for at least half an hour and be kept under inspection for about another hour After the operation is completed and the point of puncture has been sealed with collodion, a pad should be placed over the splenic area and a tight roll of bandage wound around the abdomen A 5 or 10 c c serum syringe preferably of glass with a needle of medium caliber, and about  $1\frac{1}{2}$  inches long is desirable It is important that the syringe should be absolutely dry, otherwise the parasites may become distorted and destroyed The best results are obtained when very little blood is extracted since a greater number of splenic cells are thus obtained



FIG. 63.—An endothelial cell containing about 107 *L. do. os* in the splenic puncture film. (After Dr S. Young, courtesy of JI Shanghai Sci. Inst.)

in which the parasites are situated. It may be necessary to withdraw and release the plunger of the syringe several times before the necessary material is obtained in the needle.

*Liver puncture* which appears to be less dangerous in some hands may also be employed in making a diagnosis, but the parasites are often very difficult to find and it is therefore desirable in addition to inoculate some of the fluid or blood obtained from the liver as well as from the spleen or peripheral blood on Nicolle, Novy, McNeal or other suitable media, placing the cultures at 22 C. The culture may not however develop for several weeks. In Madras the performance of liver puncture has been preferred by some to splenic puncture and it has been considered to be less dangerous. Yates has found this true in China also. Napier however does not regard it as satis-

factory as he has on more than one occasion found a liver puncture negative and on the same or following day found large numbers of *Leishman a* in material obtained from the spleen. He says that it is probable that in about 90 per cent of kala azar cases that the parasite are found in the smears from the first spleen puncture but there are cases in which the third and even the fourth spleen puncture smears are negative but in which the flagellates have been grown on culture. In partially treated cases it is usually impossible to demonstrate the presence of parasites except by cultural methods.

*Examination of the peripheral blood* may also reveal the parasite in the leucocytes but as the leucocytes are hard to find the blood should be diluted with saline solution and thoroughly centrifuged.

Artificial pustulation has been suggested by Cummins as an aid in obtaining the leucocytes in making a diagnosis. Knowles has suggested the administration of 1 cc of 1:1000 liquor adrenalin hypodermically one half hour before films of the peripheral blood are taken which seems decidedly to increase the percentage of positive findings in blood films. The Indian Kala azar Commission performed experiments to determine the effect of the subcutaneous injection of adrenalin on the blood picture in kala azar. They found in untreated cases the leucocyte response varied. In 4 cases under treatment with stibamine there was a great increase in the number of leucocytes especially polymorphonuclears and mononuclears.

Donovan, Patton, Mackie and Knowles and Knowles and Gupta have arrived at varying conclusions regarding the possibility of the demonstration of *Leishmania* in the peripheral blood by direct microscopical examination which usually necessitates careful and prolonged study of many films.

Some reports from Madras and Assam have demonstrated the presence of the organism in the blood in about 20 per cent of the cases.

Wang (1938) claims that by the use of Shortt's technique of raising a spreading slide quickly and producing straight-ended blood films in which numbers of leucocytes are gathered he found *Leishmania* in 39 per cent of 23 proved cases of kala azar.

Henderson (1936) however in the examination of 300 patients found *Leishmania a* always present in the spleen but only in 1 per cent in the blood and in 7.4 per cent in the nasal mucus.

**Culture of Blood.**—When the parasites are not found by direct microscopical examination of the blood they may sometimes be obtained by culture of the blood. Cornwall in Madras, Napier in Calcutta and Gupta have obtained good results by this method. Napier however found a few exceptions. Usually in cultures no satisfactory growth is obtained until the seventh day and it is often more satisfactory to leave the culture untouched until the tenth day. However it is not safe to discard the culture until at least 21 days of incubation have elapsed.

Young and Van Sant (1923) have recommended centrifuging a mixture of 10 cc venous blood with 50 to 70 cc of citrated Lock solution at 750 revolutions per minute then recentrifuging the supernatant cloudy fluid at 1375 revolutions and inoculating the sediment on NNN medium as they think the removal of the blood serum is an advantage. Row, Paradiso, Gupta and Adler and Theodor and others have succeeded in cultivating *Leishmania* from the blood. Adler and Theodor have shown that 0.02 cc is frequently sufficient for a culture.

**Urine.**—Shortt reported the successful cultivation of *Leishmania donovani* from the centrifuged urine in 3 out of 9 attempts. Napier however failed in 6 cases to obtain any growth from the sediment of the urine after having it stand for 48 hours.

**Bone Puncture.**—Trephining the head of the tibia and sternum puncture have also been suggested as of value in obtaining material for diagnosis particularly by Seyfarth, Jemma and Paradiso.

Recently the advantages of sternal puncture for diagnosis have been emphasized and Oelsnitz (1938) in France placed most reliance upon it.

in diagnosis and Napier (1938) who formerly thought its value was questionable now states that it has been found to be a very reliable method of diagnosis only just short of splenic puncture in the matter of accuracy and possibly safer

Also, Schretzenmayer and Lancaster (1938) state that in all cases of leishmaniasis parasites were found in the bone marrow (sternal fluid). They regard it as the most certain method of diagnosis. Kassirsky (1934) and Chung (1938) have especially employed sternal puncture for diagnosis by improved technique

Young and Osgood (1935) have reported the results of a study of sternal marrow obtained by aspiration by a modification of Arinkin's method (1927). A No. 18 gauge lumbar puncture needle 4 cm. long is used (aseptic precautions, procaine infiltration). With the point at the center of the sternomanubrial junction the needle is held at an angle of 60° with the chest wall pointing caudad and gently forced through the external lamina. Care must be taken not to let the needle jump too deeply into the sternum when the lamina gives way. The base of the needle is then depressed to an angle of 30° and the needle is gently forced in to a depth of 1 to 1.5 cm. The stylet is removed and a 2 cc. syringe is attached and 1 or 2 cc. of marrow contents is gently aspirated. If necessary the needle is rotated and the position of the tip varied until material is obtained. The material which looks like blood is expelled into a small tube containing 2 to 3 mg. potassium oxalate per cc. The needle is removed and the puncture wound sealed with collodion.

Chung advises inserting the needle obliquely at an angle of 30-40° at the midline of the sternum at the level of the upper half of the second or third intercostal space.

In the large majority of cases *Leishmania donovani* were found in every oil immersion microscopic field provided that films of marrow were satisfactory for examination.

Since 1936 Chung has performed more than 350 sternal punctures on 300 patients without an accident. Except for 2 or 3 failures which occurred during his early attempts largely due to inexperience the punctures have been very successful. By this method 171 cases of kala azar have been diagnosed. Chung concludes that sternal puncture is the best method for diagnosis of kala azar in hospitals, dispensaries and rural health stations. It is a simple and safe procedure which can be done repeatedly on all patients suspected of kala azar including those with haemorrhagic tendencies without any risk.

Cochran advises excising a lymphatic gland and making gland smears for examination but Napier has found parasites usually scanty in the lymph glands. Giraud (1938) has performed puncture of the lymphatic glands successfully for diagnosis in 4 cases of kala azar in children. Napier (1943) has found when post kala azar dermal lesions are present diagnosis can be made by examining stained smears from the cut surface of a nodule when *Leishmania* are seen often intracellularly.

**Biochemical Reactions**—Napier and Gupta (1930) have reported upon the value of a provocative dose of pentavalent antimony in the diagnosis of kala azar. They observed that the intravenous injection of an ordinary therapeutic dose of the pentavalent compound neostibosan has the effect of increasing the number of *Leishmania* in the blood of a kala azar patient. The interval between the giving of the injection and the taking of the blood examination in order to obtain the greatest number of parasites should be 10 minutes. However it has been remarked that in some cases of kala azar the parasites disappear from the peripheral circulation a short time after the first injection of antimony for treatment.

**Serological Tests**—A number of serum tests for the diagnosis of kala azar have been described. When the *Leishmania* cannot be demonstrated the diagnosis may be suggested or confirmed by one of these

**Formol gel or Aldehyde Reaction**—Add a few drops of commercial formalin to 1 cc of the suspected serum. If the quantity of blood is limited a drop of serum may be placed on a slide which is then inverted over a watch glass containing a few drops of formalin. In a few minutes the serum will solidify and become opaque. The reaction is positive in a majority of the cases of kala azar. In the early stages it may be negative and the strength of the reaction diminishes progressively during convalescence. It is usually negative in cutaneous leishmaniasis. Partial solidification may occur in some cases of tuberculosis, leprosy, syphilis and heavy malarial infections, but the serum does not usually become opaque.

Gupta regard the aldehyde reaction as the most reliable of the serum tests and Chopra (1936) reports that Napier's aldehyde reaction is positive in 83.5 per cent of kala azar cases and when the patient is cured this test becomes negative. However Faust and Meleny in their study of cases of *Schistosoma japonicum* found that a high percentage of the sera examined gave a positive aldehyde test and that the serum globulin was greatly increased and equal to that in kala azar.

**Antimony Test**—Add 2 drops of the serum to 2 cc. of a 1 per cent solution of urea stibamine or other pentavalent antimony compound. Within 15 minutes a heavy flocculent precipitate occurs in positive cases. The test may be made more sensitive by layering the serum over the antimony solution. The strength of the reaction can be estimated by the amount of the precipitate. The results of this test parallel closely those of the formol gel reaction according to Napier but with undiluted serum he thinks it less reliable than the aldehyde reaction.

Labernadie and Laffille (1929) have found that in performing the antimony test the administration of quinine (1 gm. of quinine sulphate) 2 hours before withdrawal of the blood for the test causes a precipitate to form in the sera of the persons who have no clinical symptoms of kala azar and who give negative reactions previous to the ingestion of quinine. In making this test therefore this source of error should be avoided.

The mechanism of these reactions is not entirely understood. Nattan Larrere and others (1934) have shown that two factors are involved in the formol gel reaction. The substance causing gelification is removed from the serum by dialysis while that producing opacity is retained. These investigators suggest that the two tests be combined by adding immediately to the antimony test 0.5 cc. of formalin. A heavy bulky precipitate is formed. They claim that the combined test is more sensitive and more reliable than either alone.

**Globulin Precipitation Test (Ray's Test)**—Mix one part of serum with 2 parts of distilled water. A turbidity and later a flocculent precipitate develops in positive cases. If water is poured on the surface of the serum a ring effect will be produced. This reaction is due to a marked increase in the globulin (chiefly euglobulin) content of the serum with a corresponding reduction in the amount of albumin. The reaction is not specific for kala azar however but occurs in other conditions particularly malaria. Meleny and Wu have reported very high globulin values in *Schistosoma japonicum* infections also.

Several investigators have pointed out that the erythrocyte sedimentation rate is probably greater in kala azar than in any other disease. However the estimation of this rate seems unlikely to prove a measure of any practical diagnostic value though Chung (1934) has found it markedly increased in 36 cases of kala azar that he studied.

**Skin Test**—Intradermal injections of alkalized extracts of the *Leishmania* are said to give positive reactions in a majority of the cases. The diagnostic value of these reactions and of complement fixation tests is disputed.

Animal inoculation is not useful as a diagnostic procedure since it is difficult to infect the ordinary laboratory animals with small quantities of material.

## COURSE AND PROGNOSIS

**Course**—The fever of kala azar may continue with varying degrees of severity in untreated cases for many months. The disease is usually

a chronic one in the great majority of cases, although both the adult and infantile types may show cases rapidly running to a fatal termination. The average duration of the disease in untreated cases from the beginning to their termination in 193 patients reported by Rogers in the Assam epidemic, was 7.4 months. However, he emphasizes that the disease may last for several years in the sporadic form. Muir found that the duration was seldom more than 12 to 15 months in Bengal. In some instances the disease may apparently remain dormant for long periods without obvious symptoms. The primary disease is generally considered to be rarely the direct cause of death, which is almost always due to some complication.

**Prognosis**—In earlier years the prognosis was generally considered very unfavorable. In India the mortality in untreated cases was sometimes given as high as 95 per cent and as varying from 75 to 98 per cent. The mortality in the infantile form was said to be equally high. Rogers reported that the mortality of the epidemic form in Assam in 1897 was no less than 96 per cent among the coolies of certain tea estates, in spite of their being under the care of an experienced medical man, Dodds Price and that a very similar death rate occurred in Madras City from this disease. In the more chronic sporadic type of kala azar, in the great endemic area of Bengal and Bihar it was a little less recoveries occasionally taking place but the mortality was still probably well over 80 per cent.

Some patients have recovered after having nearly died from a secondary infection complicating the disease. A leucocytosis is considered a favorable sign, while leukopenia and polymorphonuclear decrease are unfavorable signs. Unexpected recoveries are occasionally met with. The prognosis is usually poor in cases with cancrum oris in other septic infections or when the patient is exceedingly emaciated also, those cases in which there is ascites indicating fibrotic changes or those with marked intestinal disturbances. In a few cases the post kala azar dermal lesions are very resistant to treatment.

However since the recently discovered antimony treatment for the disease has been employed, the prognosis has become in many cases much more favorable. Thus Young reported the recovery of 88 per cent of 26 000 patients who were treated for several months by intravenous injections in Assam villages against a recovery rate of only 5 per cent in the same epidemic form of the disease in the Nowgong district 2 decades before. A year later the treated cases amounted to 80 000 with the result that the 1921 census showed no fall in the population in the infected areas. Napier says that probably 85 to 90 per cent of all patients who come under suitable treatment recover. Among patients treated with one of the more successful pentavalent antimony compounds the eventual cure rate is possibly 95 per cent. The proceedings of the symposium (held in China in 1931) upon the treatment of kala azar with neostibosan also emphasizes the more hopeful outcome of the disease. Struthers says that

by careful treatment with neostibosan if there are no serious complications a cure rate of from 95 to 98 per cent is to be expected

In spite of these hopeful statements the writer regards advanced kala azar as always a serious disease and the outcome of any individual case in an advanced stage is always more or less doubtful

### PROPHYLAXIS

While certain general prophylactic measures employed in India have apparently been of value in the prevention and control of the disease in the absence of exact knowledge of the usual method of transmission sharply defined prophylactic measures cannot be advised

Since the disease is to some extent one of locality houses and places believed to be infected should be avoided On the hypothesis that species of *Phlebotomus* are the vector of the malady the destruction of these minute insects and the protection against their bites seems to offer a chance of success As Patton has pointed out it is extremely difficult to carry out controlled measures against these small flies since in the majority of localities where they are common it is frequently not possible to be sure where they are breeding It is known however that they breed in moist dirt cracks crevices and holes in the ground among piles of rubbish bricks and stones in all kinds of refuse in old disused cellars on the sides of drains low down near the foundation of stone walls which are dry above earth and moist below the surface in heaps of kitchen refuse near walls and in heaps of garden refuse

The diversity and extension of these breeding places make it often difficult or impossible to deal with them satisfactorily It is important however to keep compounds of houses clear of rubbish collections of stones and bricks and where possible to close or fill up all holes cracks and fissures in the walls or close to buildings with cement mortar or tar Dark places and latrines may be smoked with sulphur fumes and put into sanitary condition and dark moist places dried whitewashed and ventilated Many of the adult flies may be killed by swatting Patton particularly recommends spraying all holes cracks and fissures with a good kerosene oil immersion He advises kylpest as being one of the most efficient It is not only a contact poison killing the flies rapidly but being an oily fluid it also repels them for quite a long time Napier believes that *Phlebotomus argentipes* is less frequently found in huts that have plastered floors

Lloyd and Napier have found that these flies prefer bovine to human blood but human blood to avian blood However we do not know whether the closer proximity of a cow would tend to attract more sandflies to the sleeping quarters or would provide a more attractive meal for the few that were already there Since ducks and fowls may attract flies they should be banished to the most distant parts of the compound and certainly not be allowed to roam freely in the compound more especially into ventilation spaces under the buildings

Although these flies are so small and delicate they can and do enter rooms sometimes at considerable distances from the ground. As however it is reported that they do not usually fly higher than 10 feet the removal of inmates to upper stories may be of some value. The height to which sandflies reach is however disputed. No gardens or cultivated ground should be permitted in the immediate vicinity of buildings and creepers should not be allowed to grow on the walls. Verandas should also not be choked with plants as is so commonly the case in tropical countries.

Napier emphasizes that *Phlebotomus argentipes* is very sensitive to smoke and is seldom found in a room where cooking is done. He advises periodic fumigation of the sleeping quarters with either sulphur cresol or crude tobacco. He points out that the range of the sandfly is a very short one and as these flies do not apparently breed out in the open effort should be made to destroy the larvae by periodically spraying with some antiseptic the possible breeding grounds and by attempting to make the soil around the hut unsuitable for breeding purposes by the addition of some cheap chemical such as lime. Some epidemiological studies seemed to show that isolating an infected area for a distance of only 300 yards sufficed to prevent the spread of the disease. However Patton (1931) found *Phlebotomus* will fly long distances especially on still nights and will sometimes enter bedrooms at least 50 feet above ground.

In districts regarded as infective it may be advisable to employ a muslin net or one with only 22 holes to the square inch since ordinary nets offer no barrier *Phlebotomus* being small. However such nets are at the time of the year when infection seems most likely to occur almost unbearably hot.

Deterrents are sometimes of value. Several lumps of camphor placed in the bed are said to be sometimes effective. Balfour has recommended a repellent ointment for application to the skin as being most efficacious. This consists of oleum anisi eucalypti and terebinth each minims 3 and lanolin ounce 1. Choyce has recommended 5 per cent thymol made up into a firm ointment and rubbed into the skin as an excellent prophylactic measure against *Phlebotomus*.

Air currents have a marked effect on sandflies and Whittingham has shown that one of the most effective ways of ridding quarters of these pests is to create a strong current of air by means of electric fans.

The cases of kala azar should of course be dealt with as infectious and segregated protected from bloodsucking arthropods and treated with antimony compounds. In Mediterranean areas and other localities where canine leishmaniasis is present dogs and especially infected dogs should be destroyed. Especially in the endemic districts dogs should be kept away from association with children as well as adults. In India general measures that apparently have been of advantage in former years are the segregation of the sick, burning of the houses, clothing and even furniture and the provision of new huts.

Good results have also followed the actual treatment of cases on a large scale. Rogers in his campaigns against the disease emphasized first

the measures possible to prevent new infections arising and secondly the influence of the greatly improved treatment of the disease in diminishing the sources of infection

Young has also recently described the methods employed in Assam in connection with the prevention of the disease. When large numbers of cases have been discovered in a village it is reported as an infected area. The inhabitants are removed from the infected site each family being classified under one of three groups namely infected contact and free. Each of the groups is located on separate sites. The infected ones are promptly treated while the remaining are placed under observation.

Napier also emphasizes two principal lines of attack namely the segregation of the sick and contacts and treatment of the sick.

Kundu (1931) who has been in touch with kala azar work in Assam since 1917 states that up to 1920 segregation of infected persons, evacuations of sites and houses and burning of huts and clothes were apparently the only possible methods adaptable to prevent the spread of the disease. Owing to the hardships inflicted by these methods there was an ever increasing tendency on the part of the population to conceal cases. Since the introduction of antimony treatment there has been a diminished incidence and the death rate has been reduced from 39.3 in 1920 to 7 in 1928.

### TREATMENT

Prior to 1915 many drugs had been tried in kala azar without any of them showing any definite efficiency or producing any appreciable reduction of the high mortality of the disease. Intravenous injections of tartar emetic were apparently first employed by Martin and LeBoeuf (1908) in the treatment of trypanosomiasis and attention to such treatment was emphasized by Kerandel's report of curing himself of trypanosomiasis in this way. Subsequently the method was employed by Vianna and Machado (1913) in the successful treatment of cases of Brazilian cutaneous and mucocutaneous leishmaniasis. Caronia and diCristina (1915) then employed intravenous injections of tartar emetic for the treatment of infantile kala azar in Italy. Of 8 cases which they treated 5 were cured, 2 were recovering and only 1 had died of complicating nephritis at the time the report was made. These favorable results were confirmed and extended by Rogers (1915), Muir (1915), Price (1919) and Knowles (1920) in India who thoroughly demonstrated the great value of the antimony treatment. Rogers especially emphasized it as a specific cure for the disease.

More recently the introduction of the organic pentavalent compounds of antimony in which the antimony is attached directly and not as in tartar emetic through oxygen and carbon have given even more favorable results. It has been well recognized that potassium and sodium antimonial tartrates when inoculated intravenously may produce toxic and inflammatory symptoms and not infrequently give rise to pneumonia the unfavorable results apparently being due to the liberation of antimony



trioxide by the serum alkali. Pneumonia has constituted one of the most frequent causes of death during treatment with tartar emetic.

On the other hand the pentavalent compounds are not only much less toxic, but also give much more rapid results in treatment. Many workers who have recently investigated the clinical efficiency of the antimony preparations in kala azar, as Napier, Rogers and Kundu in India and Struthers, Morgan and Woods and Bell in China, all emphasize the advantages of the pentavalent compounds over the sodium and potassium tartrates of antimony. However, Caronia (1930) in reviewing the progress of the treatment of kala azar from the time of the introduction of tartar emetic in 1915 states that he is convinced that tartar emetic remains the best remedy for the disease when it is possible to administer it intravenously. The expense of the pentavalent compounds of antimony is obviously considerably greater than of the inorganic salts.

The preparations of antimony which have been particularly employed in the treatment of the disease are the sodium and potassium tartrates of antimony, stibenyl (stibacetin), urea stibamine (stiburea), stibamine glucoside or neostam, stibosan, neostibosan and solustibosan.

**Sodium and Potassium Tartrates of Antimony**—Sodium and potassium tartrates of antimony have been extensively used in a 2 per cent freshly prepared solution administered intravenously. The dose recommended for an adult to begin with is 2 cc of the solution containing 0.04 gm of the salts. The dose should be increased by 1 cc at each administration up to a maximum of 5 cc or 0.1 gm. Subsequently the dose should be 5 cc on each occasion. The injections should be made on alternate days throughout the course of treatment and continued until 4 grams of the salt have been administered. In infants of 3 years, the first dose should be 0.5 cc of a 2 per cent solution and increased to 2 cc as a maximum. For a child of 12 years 1 cc should be at first injected and increased to a maximum of 3.5 cc. For other ages the dose should be proportionate.

*Symptoms of intoxication* are not uncommon. They appear immediately after injection though sometimes at later periods. They may consist of metallic taste, headache, giddiness, sore throat, cough, nausea, tachycardia, diarrhoea, muscular stiffness and great debility. The drug may produce severe fatty degeneration of the heart, liver and kidneys. The most common toxic symptom after the injection is a fit of coughing. This is a very frequent occurrence and is not usually of great significance. In some instances, however, the cough may be so severe that the patient eventually vomits. If this takes place it is always an indication that the dose should not be increased. Nausea is also an indication against any further increase in the dose. If actual vomiting occurs the next dose should be reduced and only increased again when tolerance has become established. Both vomiting and coughing may be induced by giving injections on a full stomach or by injecting the solution too rapidly. Codeine, gr  $\frac{1}{4}$  or liquor adrenalin minimis 10 given intramuscularly about 20 minutes before the injection of the antimony salt will often reduce the tendency to cough.

The extreme frequency of the occurrence of respiratory complications during the course of treatment with the antimony tartrates led to the suspicion that the condition was actually caused by these injections and the extreme rarity of these complications among patients treated with the pentavalent compounds has now confirmed this suspicion. Napier found that pneumonia is one of the most distressing complications of

treatment and frequently carries off a patient who has completed about half his course of antimony and is progressing favorably to convalescence. A number of deaths have been reported by different observers as due to the drug. Rogers encountered at least 3 deaths. Christopherson 2 and Low and Knowles one each and a number of deaths have been reported in Italy among children due to poisoning by the drug in the doses recommended.

The drug has some depressing action upon the heart and very marked slowing of the heart is met with in some cases towards the end of the course of treatment. When this occurs it is certainly an indication that the treatment should be suspended. During the administration of therapeutic doses the fall of blood pressure is usually very slow and only transitory. Chopra however found that large doses when given to cats especially if given rapidly cause a pronounced and lasting fall of the blood pressure. Any kidney disturbances in man are likely to be aggravated by the antimony tartrate injections.

Mainzer and Krause (1940) in the study with the electrocardiogram of 12 cases undergoing treatment with tartar emetic found in 7 considerable alterations and in 3 they were markedly pathological. The effect of the changes was parallel to the degree of bradycardia and was considered as resulting from intoxication of the heart muscle due to tartar emetic.

Severe joint and muscular pains are very common complications of treatment but fortunately seldom occur except towards the end of the course of injections. They usually come on some 4 or 5 hours after the injection and according to Napier last for anytime up to 12 hours. He has found that by giving 20 gr of a purg about half an hour before the pains are expected to commence their severity can be diminished considerably.

Irritating papular eruptions occasionally occur. They do not usually disappear until the injections have been discontinued.

A sharp rise of temperature may occur in some of the cases following the injections. However under the treatment the temperature usually falls steadily with the exception of a possible temporary rise on the day of an injection. Often after about the fifth injection the patient is entirely free from fever. In most cases the temperature should have become normal in 1 to 3 weeks. Even then however an intermittent rise may occur on the days of the injections. Napier believes that a sudden sharp reaction of fever in a patient in which the temperature has subsided usually indicates that too big a dose has been given. Increase in the size of the spleen and liver may also occur as a result of the injections of antimony.

**Curative Dose**—While the amount varies in different cases Napier found the maximum curative dose of the tartrates of antimony to be about 4 gm for every 100 lbs of body weight. However in many cases in which the improvement is evident in a short time a total of 2.55 gm in 30 injections is sufficient. In still more resistant cases full 4 gm in 45 injections should be administered.

**Pentavalent Preparations**—Of the pentavalent preparations of antimony neostibosan, neostam, solustibosan and ureastibamine appear to be the most satisfactory. Ureastibamine (stiburea) is a compound of urea and stibamine (*p*-aminophenylstibinic acid) which was introduced by Brahmachari in India. According to Indian reports it has proved to be a very efficient preparation. However it has been reported that it is apt to undergo chemical changes if exposed to the air. Neostam is a stibamine glucoside.

**Neostibosan** (Boyer 693) (para amino phenyl stibinic acid) combined with an amine would appear to be a more satisfactory compound for treatment. It contains 40 per cent of metallic antimony and is comparatively non toxic. Different preparations of this drug have been found to differ considerably in their efficiency. More recent preparations have been prepared and sold under the term Bayer 693B. It may be given in a strength of 25 per cent and injected either intravenously or intramuscularly. The initial dose for an adult is 0.1 gm. the second dose 0.2 gm. the third 0.3 gm. The doses may be given daily. About 10 injections are required for an average case and it is said that a total of 7-4.0 gm. is usually necessary to effect a cure. The special advantage of this drug is that it may be given intramuscularly to children. Antimony preparations administered intravenously to children are apt to be followed by bronchial pneumonia and Neumann reports that since using neostibosan intramuscularly bronchial pneumonia has been much less frequent. This drug is now 1944 prepared by the Winthrop Chemical Co.

Woods and Bell have published a report of 2539 cases of kala azar treated during the first 11 months of 1930. Of these 1097 were patients in the hospital while the remainder were quartered near the hospital and came daily for treatment. With few exceptions all were treated with neostibosan. For an adult of 120 lbs. weight and in good condition the initial dose was 0.1 gm. This was increased by increments of 0.05 gm. until a maximum of 0.3 gm. was reached for hospital patients and 0.25 for out patients. The injections were given usually on alternate days up to a total of 3 or 3.25 gm. Of the 2539 cases 1914 were reported as cured, 473 as still under treatment, 43 as having died and 109 as having left the hospital before treatment was completed.

**Solustibosan**—A new drug Solustibosan (Bayer 561), a sterile isotonic neutral solution in water has recently been introduced which is reported as superior in efficacy to neostibosan.

Kikuth and Schmidt (1938) who have used the European hamster for testing the value of different drugs and controlling the result of the infection by liver puncture recommend solustibosan especially, the toxicity of which is lower and larger amounts of antimony can be administered than is the case with other pentavalent compounds. One cc. of this preparation contains 20 mgm. antimony and 6 cc. corresponds to 0.39 gm. neostibosan. Dose 2 cc. for each kgm. body weight.

Struthers Napier and Yates (1939) have also used this drug solustibosan with good results.

Wang (1938) has reported that in the treatment of experimental kala azar much more antimony can be given in the form of solustibosan than of neostibosan but more of the drug is required to complete the cure. Daily injections are said to be less effective than biweekly or triweekly. It may be given intravenously or intramuscularly. Later it was shown that larger doses of solustibosan (10-1 cc.) could be given with safety (Kikuth 1943) hence a concentrated solution of it has been prepared which contains 100 mgm. of pentavalent antimony per cc. Kikuth states that in spite of the solution being hypertonic it is absorbed without irritation from the tissues. An oil suspension of solustibosan in which 1 cc. contains 54 mgm. of pentavalent antimony has also been prepared.

It is claimed that both of these preparations are more satisfactory for treatment than the old solustibosan. Lozano Morales believes the con-

concentrated solution of solustibosan is most satisfactory. He treated five cases in children in Spain without evidence of toxicity.

**Choice of Preparation**—It is perhaps unfortunate that many of the reports of the results of treatment with the different antimony preparations omit references to the number of deaths.

In 1924 Napier reported a 14.4 per cent death rate in a series of 139 cases treated with sodium and potassium antimony tartrate while in a similar series of 35 cases treated with sodium antimony tartrate the death rate was 22.8 per cent. However these figures are in marked contrast to those obtained in 167 cases treated by him with 6 different pentavalent compounds in which the death rate was only 4.2 per cent. Struthers and others in China have also had much more favorable results with the pentavalent compounds.

Napier points out that the disadvantages of the pentavalent compounds are few. It seems possible that post treatment jaundice is a little more frequently encountered in patients who have been treated with them and with some an anaphylactic like group of symptoms may appear suddenly towards the end of the course of the injections. He believes that there are practically no special advantages in using the antimony tartrate salts and that the only thing that stands in the way of the total abandonment of their use is the relatively high cost of the pentavalent preparations. Taking 3 gm. as the dose necessary to effect a cure he points out that in India the cost of curing one patient will be 3 pence if sodium antimony tartrate is used and at least 25 shillings if one of the pentavalent compounds is used.

Napier in comparing the value of the different pentavalent compounds points out that the mean number of injections prior to the cessation of fever of each series treated with the different preparations was as follows: stibosan 5.6, stibamine glucoside 5.4, urea stibamine 5.1, No. 693 4.57 and amino stiburea 4.48. Stibosan showed the highest death rate and No. 693 (neostibosan) the lowest. He emphasizes that in view of the fact that the cases treated with neostibosan were not in any way selected and that quite a number of very debilitated patients were included in the series it is rather remarkable to be able to report upon 61 cases without a single death. Jaundice which is a common sequel to the treatment with most of the preparations appears to be rare with neostibosan. Stibosan he believes is the most stable compound while amino stiburea is possibly the most powerful in its action. However neostibosan is the most innocuous and if its curative value is less it is only slightly less than that of amino stiburea and he believes that it will prove the most useful drug in the treatment of kala azar. Also Struthers, Bethell, Turner, Morgan and Woods and Bell in China evidently consider neostibosan to be the most satisfactory pentavalent compound for treatment. In Struthers' series of 87 cases 3 died, of Turner's series of 72 died, while of Woods' and Bell's series of 539 which with few exceptions were treated with neostibosan 43 died.

Ermen (1938) emphasizes that the activity of the pentavalent compounds is associated with a leukocytic response in the blood and the beneficial action in kala azar is probably due to the double action, one on the parasites and one on the patient. He believes the trivalent compounds like foudadin are inactive. However some favorable reports from the use of foudadin have been made.

**Technic of Injections.**—Nothing with reference to the technic of performing the intravenous injections with the different antimony preparations need be stated except to emphasize that care must be taken that the point of the needle be introduced clearly into the vein and not into the wall of the vessel as any escape of the fluid into the tissues will produce acute inflammation. Veins of the elbow are usually most suitable. However in very young children and in a few adults the veins are so small or so deeply buried that it is almost impossible to puncture them. In Italy some injections have been made into the external jugular vein but fatal toxic results have been recorded in

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**Method of Administration**—Since the introduction of antimony in treatment all possible methods of administration have been tried. The intravenous injections are generally by far the most satisfactory. Under certain circumstances intramuscular injections may have to be employed, and in some cases intravenous injections may cause severe constitutional symptoms. Intramuscular injections are superior to subcutaneous administration as the latter almost invariably produces cellulitis which may lead to abscess formation.

Only the more recent pentavalent compounds are satisfactory for intramuscular injections. Some preparations cause considerable pain at the time of injection, and others cause less pain at the time, but considerable swelling and pain later. The fever may last for a week.

The gluteal region seems to be the most satisfactory site but the deltoid muscles may also be suitable and repeated injections of 0.1 gm. of the drug may be given. This amount may be given in 2 cc. of a 5 per cent solution. For a child this dose may be sufficiently large to effect a cure in 10 to 12 injections but for an adult it may be necessary to give daily injections and to give at least 25 of them before a cure is obtained. Napier has more recently treated 20 cases intramuscularly with 0.3 gm. of neostibosan in 25 per cent solution in distilled water. In only one instance out of about 200 injections was there inflammation followed by abscess.

None of the compounds that have been produced is suitable apparently for oral administration. The tartrates are very irritating if taken in anything but minute doses and the pentavalent compounds are apparently not absorbed. Napier gave 2 patients one gram of stibenyl a day for 14 days without obtaining any clinical improvement in either case. The urine at no time contained more than a trace of antimony.

*Napier does not believe that rectal administration is successful.*

Smyly (1927) treated 3 cases with rectal injections of tartar emetic—0.04 gm., increasing up to 0.3 gm. in 100 cc. of normal saline. The injections were well retained and antimony was found in the urine in all cases, but the antimony was not absorbed in sufficient quantities to be effective. However a few reports of successful treatment of children by rectal injections have been made.

**Disturbances Associated with Treatment by Pentavalent Compounds**—Vomiting is one of the most common disturbances. The patient usually vomits within about 20 minutes after receiving the injection. The vomiting may be preceded by giddiness and nausea. If when premonitory symptoms appear the patient remains quietly at rest in bed actual vomiting is sometimes avoided. With stibosan Napier found this complication rare except when the injections were pushed beyond the usual maximum dose. With ureastibamine, amino stiburea and neostibosan he found that vomiting occurred in approximately 10 per cent of the cases. In cases which show a tendency to vomit the dose should be kept down to about one half the usual maximum dose and the increase in amount should be made very cautiously.

Patients also sometimes suffer from diarrhoea which may become severe towards the end of a course of treatment. The patient may become collapsed and one of

Napier's patients died from the effects. However if the treatment is discontinued the condition usually improves.

Symptoms resembling those of anaphylactic shock sometimes occur quite suddenly. They have been noted particularly after the sixth or seventh injection when the patient had been receiving the maximum dose for the last few injections. The patient's face may become puffy and an urticarial rash appear all over the body, the voice becoming husky and there may be considerable difficulty in breathing. Sometimes the patient becomes collapsed, the pulse being imperceptible at the wrist or there may be violent diarrhoea and vomiting, the patient becomes cyanosed at times breathing stertorously and remaining unconscious for some minutes.

All these unfavorable symptoms usually disappear within 2 hours, but the puffiness of the face may last for 4 hours or longer. While these symptoms may be alarming Napier records no deaths from them, but as further administration of the smallest dose may lead to a recurrence it is best to abandon treatment with the particular compound altogether and to choose some other pentavalent preparation.

In a few instances after treatment with stibosan and urea stibamine symptoms of acute congestion of the liver have been noted, the organ becoming markedly enlarged and the patient complaining of severe pain in the hepatic region. There also may be a return of fever if the temperature has fallen to normal. In other instances the spleen becomes acutely enlarged. If the treatment is discontinued immediately the symptoms will usually subside.

**Relapses**—A relapse in kala azar after insufficient treatment is not very uncommon. When a relapse occurs the patient must be given a further and much more thorough course of treatment. Care should be taken not to confuse fever due to malaria or other infectious disease as a relapse of the kala azar. A very definite enlargement of the spleen usually accompanies fever that is due to a relapse and where possible spleen puncture should be done to confirm the diagnosis.

Mitra believes that relapse may be due to parasites concealed in the bone marrow or spleen where the circulation is retarded and which escape the action of the drug.

In some patients the disease is much more resistant to treatment than in others. Reports have been made of certain cases of kala azar that are uncontrolled by any of the antimony preparations. These cases usually go on to a fatal issue in spite of prolonged treatment over many months and death usually occurs through some complication. Other patients show an intolerance for antimony apparently due to an idiosyncrasy for the drug.

Some writers think that insufficiently treated cases that have relapsed are then more resistant to antimony treatment but Napier is opposed to this view.

**Criteria of Cure**—It is important to recognize when the patient is cured and when it is safe to discontinue antimony. Napier and Halder have found that a total relative dose of 3 gm. about 30 injections in an average adult will effect some 80 per cent of cures and they believe that prolongation of treatment beyond this point will produce only a very slight increase in the rate of cure. However as they found no evidence that a prolongation of treatment up to a limit of about 6 gm. is detrimental to health a very thorough course of treatment is desirable in private patients.

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Napier does not believe that rectal administration is successful.

Smyly (1927) treated 3 cases with rectal injections of tartar emetic—0.04 gm increasing up to 0.3 gm in 100 cc of normal saline. The injections were well retained and antimony was found in the urine in all cases but the antimony was not absorbed in sufficient quantities to be effective. However a few reports of successful treatment of children by rectal injections have been made.

**Disturbances Associated with Treatment by Pentavalent Compounds**—Vomiting is one of the most common disturbances. The patient usually vomits within about 20 minutes after receiving the injection. The vomiting may be preceded by giddiness and nausea. If when premonitory symptoms appear the patient remains quietly at rest in bed actual vomiting is sometimes avoided. With stibosan Napier found this complication rare except when the injections were pushed beyond the usual maximum dose. With ureastibamine, amino stiburea and neostibosan he found that vomiting occurred in approximately 10 per cent of the cases. In cases which show a tendency to vomit the dose should be kept down to about one half the usual maximum dose and the increase in amount should be made very cautiously.

Patients also sometimes suffer from diarrhoea which may become severe towards the end of a course of treatment. The patient may become collapsed and one of

solutions may cause severe late toxic effects on the liver kidneys or pancreas even after completion of a course of treatment. The dose is 10 milligram (0.001 gram) per kilogram of body weight (maximum adult dose 0.15 gram) every other day for 15 injections. Repeat this course of treatment *only* if cure is not obtained and *after interval of one month*. Some cases show fall of blood pressure and syncope after first or second injection. This may be prevented or relieved by injection of a small dose of epinephrin. The administration of calcium and glucose during treatment may protect the liver from damage. (From personal communication Warrington Yorke 1942) \*

**General Treatment**—The general treatment of the disease should be symptomatic particularly so with reference to complications. In patients who are debilitated and show cardiac disturbances the administration of digitalis should be considered. If however the pulse rate becomes unduly slow it obviously should be discontinued. If the patient has come from a malarious district malarial infection should be excluded or quinine should be given daily otherwise a malarial attack may occur while the patient is undergoing antimony treatment. As malarial parasites are sometimes difficult to find in the blood in chronic cases of the disease particularly if some quinine has previously been taken the administration of a short course of quinine in native patients would seem wise.

No special recommendations need be made with reference to diet unless intestinal disturbances exist and the patient may in general be allowed to eat whatever he cares for provided that the diet is nutritious and he is able to digest it. During the febrile periods of course only light diet should be given.

The accompanying anaemia may require treatment with iron and such treatment may be supplemented by liver extract.

**Treatment of Complications**—In cancrum oris there is little hope of curing the condition by local measures until the general condition of the patient improves. Strong antiseptics should be avoided as the resistance of the tissues is lowered and they are easily destroyed.

Cases complicated with malaria or ankylostomiasis should receive specific treatment. In China and in parts of India almost every patient has more or less severe infection with hookworm and the return of the blood picture to normal can hardly be expected unless iron is administered and as long as the patient harbors ankylostomes. Pneumonia is a frequent and very serious terminal affection however a fair proportion of the patients affected with it recover. Sulfapyridine and serum treatment should be considered when serum is available.

With reference to the treatment of disturbances caused by antimony injections these usually pass off when the antimony treatment is discontinued. Sometimes the diarrhoea will require treatment with bismuth and opium. The anaphylactic like symptoms are usually relieved by the administration of adrenalin. Strychnine and digitalis may be necessary to combat the collapse. The jaundice which sometimes follows treatment usually requires no special attention and disappears gradually of its own accord.

Napier (1943) is pointing out the toxicity notes neuropathy subjective disturbance over parts of the trigeminal nerve area in twenty cases.

Rogers and Napier give as clinical criteria for discontinuing treatment the complete cessation of fever and its continued absence for a considerable period particularly if accompanied by a substantial gain in weight approaching nearly to the normal disappearance of the spleen beneath the costal margin or reduction of it in size by several inches the disappearance of the leukopenia with increase of the total leucocytes Rogers advises that it is well to see the patient occasionally after recovery to make sure that no relapse occurs

From the laboratory standpoint the performance of spleen punctures with cultures of the splenic blood and absence of the parasite both microscopically and after 3 weeks of cultivation afford the most reliable evidence of a complete cure A simple microscopical examination alone cannot be relied on because very few parasites may be present particularly towards the end of recovery

Although the aldehyde test often becomes negative after recovery as emphasized by Lereboullet Chabrun and Baize this negative reaction is not generally regarded as a very reliable criterion of cure Napier points out that the serum of a patient who has just completed his course of treatment and is cured very often still gives quite a strongly positive reaction Struthers in China regards the aldehyde test as of no value as evidence of a cure

Lloyd Napier and Paul in the study of the serological control of the treatment of kala azar found that if treatment is effective the serum proteins resume their normal values approximately 120 days after treatment has been begun The high globulin low albumin condition of the blood serum and total low proteins are believed by the authors to be favorable to the growth of the parasite

Chopra Chaudhury and De have studied the changes in the physical properties of the serum in cases under treatment with pentavalent compounds and the relation of the formol gel reaction The viscosity of the serum and the rate of occurrence of gelation and complete opacity of serum under the influence of the formalin was observed They found that viscosity of sera does not decrease for about 2 months after treatment has been commenced The time of complete opacity begins to increase after about 10 to 15 days whereas the time of gelation increases after about 20 to 30 days They suggest that the protein responsible for gel formation is not euglobulin

Brahmachari has made observations after intravenous injections of antimony of the antimony laden cells of the spleen of mice infected with *Leishmania donovani* It was found that when metallic antimony was injected intravenously into healthy mice it was picked up inside the spleen by cells that contained *Leishmania* and an antimony compound developed inside the cells that killed the *Leishmania* In certain cells in the spleen the antimony was found in a diffuse state probably the stage in which the solid finely divided antimony is converted into colloidal particles before passing into complete solution Degenerated *Leishmania* were sometimes observed in cells containing particles of antimony

**Stilbamidine**—Recently favorable reports have been made in treatment by the new preparation 4,4-Diamidino Stilbene (See chapter on Trypanosomiasis Page 206) Napier has reported the treatment of 108 cases in India 98 of which were cured after 8 injections Adams and Yorke and Napier and Sen (1941) have also reported good results from its use in India In the Egyptian Sudan and some adjacent areas leishmaniasis is very resistant to treatment with antimony preparations (Cole Cosgrove and Robinson (1942) and Mohammed Sati (1942)) British investigators have recently reported that antimony resistant cases show a good response to intravenous injections of stilbamidine isothionate (4,4-diamidino stilbene isothionate) The drug must be used in a freshly prepared solution in 10 c.c. of sterile distilled water without heating The water must be neutral or very slightly acid (pH<sup>6.8</sup>—7.8) Old

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(For additional references see pp 319-320)

For the treatment of post kala azar dermal leishmaniasis Napier has found that the intravenous injection of antimony is the only treatment that has any effect on the lesions. In some instances the cases proved refractory to treatment.

Kirk and Hamad Satı (1940) have observed a punctate cutaneous eruption in treated cases of kala azar in the Anglo Egyptian Sudan. While the question of the influence of antimony in the development of the rash was considered in 5 cases of 20 who did not receive antimony during treatment the rash was also observed.

Brahmachari in an attempt to shorten the period of treatment of cases of dermal leishmanoid found that intravenous injections when combined with local inunction of the skin lesions with metallic antimony gave more satisfactory results than those following intravenous treatment alone.

Napier believes that treatment should certainly not be withheld on account of pregnancy and that at whatever stage of pregnancy the patient is seen treatment should be commenced without delay. He has treated a number of cases in pregnant women who have subsequently given birth to healthy children.

Splenectomy in general would appear not to be justified as a therapeutic measure in visceral leishmaniasis. Cases which have been reported by Wylie De Souza and Olmer from Europe and Asia would indicate that there are few cures and a relatively high mortality due to shock.

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In India noteworthy centers of infection are Lahore Multan and Delhi and in earlier years Manson cited that from 40 to 70 per cent of Europeans residing in Delhi suffered from this affection which led to the name Delhi boil. In the Punjab Mesopotamia and Asia Minor where oriental sore is most frequently met with kala azar is rare.

In Asia and Africa the terms Aleppo boil and Biskra button are an indication of the prevalence of the affection in these localities in earlier years.

It has been reported by reliable observers that in Bagdad nearly every child is attacked and that it is quite exceptional for any native to attain maturity without having had one or more of these sores and that every woman in Bagdad has on her face marks of the ravages of this disease.

In the western hemisphere the writer has observed its prevalence in Brazil and Peru. It also occurs frequently in Guiana and Paraguay. Cases have been reported from every country of South America except Chile and Patagonia.

Da Silveira and Pupo in Brazil have particularly emphasized its prevalence. Da Silveira found that 50 per cent of 15,000 patients seen at São Paulo were infected. Pupo reported that 13 per cent of the patients in the hospital for venereal and skin diseases in São Paulo during 1914 were under treatment for leishmaniasis and by 1919 the figure had reached 48 per cent its prevalence affecting the labor conditions.

Fuchs (1929) also found leishmaniasis with great frequency in São Paulo and in Bolivia.

However in parts of Amazonia it is not nearly as frequently seen as it is in the Andean regions farther to the west though Walker Shattuck Wheeler and Strong Da Matta and Chagas have reported cases. Cases have also been reported in Argentina Paraguay Central America and Mexico (Bernasconi Migone Darling and Connor Chacon Seidelin Shattuck).

In the United States only sporadic cases of oriental sore have been observed in individuals who have returned from endemic regions. Andrews reporting a case also cites 9 other instances which were encountered either in the United States or in Canada.

**History**—Oriental sore or Aleppo boil has been recognized and described since the latter part of the 18th century. Russell (1756) and later several other physicians found it to be endemic in Aleppo and gave accounts of it under the name Aleppo boil.

Hirsch (1886) has given a most interesting account of the history of the affection and of its infectious nature. He points out that some of the earlier experimenters such as Polek Groschl Vandyke Carter and Wortabet who had tried the experiment of inoculation on themselves did not succeed in producing the disease and therefore pronounced against its communicability. Others like Fleming (1868) Weber and Murray who had also experimented on men (Fleming on himself) obtained a positive success and it was believed that there was no well grounded doubt of the communicability of the boil.

## Chapter VI

### CUTANEOUS LEISHMANIASIS

**Synonyms**—Oriental sore Bouton d'Orient Bouton de Biskra, Aleppo Bagdad or Delhi boil Espundia Uta Bubas Forest yaws Chiclero ulcer American Leishmaniasis

*Cutaneous leishmaniasis* or oriental sore may be defined as a specific nodular affection or circumscribed ulceration of the skin or mucous membranes of exposed parts caused by *Leishmania tropica*. It is endemic in many tropical and subtropical countries in various parts of the world. The nasopharyngeal or oral forms occur particularly in a number of forest regions in South America and have been described by some writers under the term American leishmaniasis. Numbers of imported infected cases have been observed in the United States. The condition is inoculable and may be conveyed by direct contact between infected and healthy individuals. The Sergents and Adler and Theodor and others have shown that the infection may be transmitted also by the inoculation of crushed sandflies *Phlebotomus* which contain the flagellate forms of the parasite and there is considerable other evidence which suggests that natural infection of man may occur through *Phlebotomus*.

#### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**Geographical Distribution and Prevalence**—The disease is epidemic in many tropical and subtropical countries in both the Western and Eastern hemispheres. It occurs especially in certain localities in south east Asia from Asia Minor Syria Palestine Armenia Mesopotamia and Arabia Persia the Caucasus the southern part of Russia Turkestan eastward to the Punjab and northwest province of India as far as Cambay near Bombay. In China it has been found especially in Hunan.

It is also very common in the Mediterranean littoral and adjacent territory Asia Minor Syria Palestine. In Africa Morocco Tunis Algiers the Sahara Egypt the Sudan Abyssinia the French Congo (*Niger District*) *District of Lake Chad* and *Nigeria* are endemic areas. On the West Coast it has also been reported to be not uncommon as far south as Angola.

In Europe the disease is endemic in the Mediterranean Islands of Sicily Cyprus Crete and Sardinia and in recent years it has been introduced by returning soldiers or immigrants into southern Italy Spain and Greece. In Italy Vanni (1938) reported an outbreak in Abruzzi and Monti and Pozzi (1939) have observed a large focus in Forlì Italy. In one municipality 60 cases were found. Cases have also been reported in the south of France.

differences between *Leishmania braziliensis* and *Leishmania tropica* can be found either in the forms in the tissues or in cultures nor can any differences be demonstrated by experiments upon animals. It is also doubtful if they can be distinguished by serological tests. It would appear that in much of the earlier work in differentiation by the agglutination test the experiments were not fully controlled. The details of such experiments have been given in the consideration of the etiology of kala azar where the most recent work is referred to. Da Cunha (1940) believes *L. infantum* and *L. chagasi* are identical as shown by inoculation of hamsters, monkeys and dogs and serological tests. However it is by no means certain that only a single type of organism causing cutaneous leishmaniasis exists and for clinical and epidemiological reasons it may be advisable to retain the term *L. braziliensis*.



FIG. 64.—Smear from a lesion showing *Leishmania*.  $\times 100$ . (From May.)

Thomson and Balfour (1920) described a type of cutaneous leishmaniasis in the Sudan in which the lesions were nodular and showed no tendency to ulceration. Although the organism was morphologically indistinguishable from *Leishmania tropica*, Brumpt presumed the disease was distinct from oriental sore and proposed for the parasite the name of *Leishmania nilotica*. However subsequently the writer observed a somewhat similar cutaneous lesion in Amazonia and also Napier and others have described such lesions under the term of post kala azar dermal leishmaniasis; hence the name *Leishmania nilotica* should be regarded as a synonym.

The close resemblance of *Leishmania tropica* to *Leishmania donovani* led Manson to suggest that the relationship between oriental sore and kala-azar might be compared to that of vaccinia and variola. He also based this view upon the immunity produced by one attack of oriental sore against further infections of the same disease and upon the well recognized dissimilarity in the distribution of the two conditions. In India, for example, in the localities in which kala azar is common, oriental sore is rare.

Köhle and Lancelaux reported in experimental monkeys and dogs that an animal which had recovered from oriental sore was immune to this condition but not to kala



Seriziat as early as 1875, and Laveran in 1880 suggested that it might be communicated by winged insects

With reference to the American or nasopharyngeal form the disease as uta has apparently existed in Peru since prehistoric times (Tamayo Palma)

In 1875 Van Dyke Carter thought that a fungus was the cause of the disease while in 1886 Riehl described as the cause a capsulated micrococcus which occurred particularly in the cytoplasm of large epithelioid cells

The parasite of oriental sore was first definitely discovered and described by J H Wright in 1903, in the study of an ulcer of a child in Boston the patient having come from Armenia where the infection was presumably contracted Wright named the parasite *Helcosoma tropicum*, suggesting that it was a protozoan, and allied to the microsporidia Subsequently it was recognized that the parasite was a species of the genus *Leishmania* and the correct name became *Leishmania tropica* When the parasite was cultivated by Nicolle in 1908 further confirmation of its zoological position was obtained The possible observation of this parasite by other investigators, as Cunningham Marzinowsky and others has been considered under the heading of the description of the etiology of kala azar p 234

In South America Lindenberg (1909) and Carini and Psranhos (1909) reported finding leishmania in ulcera de bauru of the skin in Brazil and Splendore (1911) found the parasite in one type of buba brasileira with lesions in the mouth and nose Escomel (1911) also found the parasite in espundia while the Harvard Commission in Peru (1913) found leishmania apparently identical with *Leishmania tropica* in the lesions of uta Nattan Larrier and Touin (1909) also found *Leishmania* in an ulcerating lesion of the skin in Guiana termed pian de bois

### ETIOLOGY

The parasite of oriental sore *Leishmania tropica* cannot be distinguished morphologically from *Leishmania donovani* See Fig 54 The morphology of the latter parasite has already been described in detail on p 236 and will not be repeated here In many cases of infection with *Leishmania tropica* in South America the lesions are limited to the skin as is generally the case in Asia and in European endemic areas However in a number of cases in South America the lesions involve only the nasopharyngeal mucosa Such mucous or mucocutaneous lesions are often very severe and extensive For these reasons certain writers have referred to the nasopharyngeal forms under the term American leishmaniasis and it has been suggested that the parasite in such cases is perhaps not identical with *Leishmania tropica*

From 1911 on Vianna Escomel Rebagliatti Laveran and Nattan Larrier, Velez Chagas and others have described these forms of the disease and have suggested various names such as *Leishmania brasiliensis* *L. chagasi* and *peruviana* for the causative organism No morphological

probable that *Leishmania tropica* grows more vigorously and luxuriantly. However, there is much variation with different strains.

To obtain cultures from an oriental sore it is advisable and often necessary to secure material free from bacteria, though it has been sometimes observed that *Leishmania tropica* will flourish more readily in the presence of contaminating micrococci than will *Leishmania donovani*. In ulcerating varieties this often can be done by carefully sterilizing the skin at the edge of the ulcer with iodine or other antiseptic, making puncture with a needle or sharp knife and drawing off material by means of a sterile pipette. This is then blown into the liquid at the bottom of the tube of NNN or other suitable medium. Flagellates if present may be detected in the tubes in from 3 days to 3 weeks according to the number of organisms introduced.

### EPIDEMIOLOGY AND ENDEMICITY

Although oriental sore may occur in countries where kala azar is endemic, its distribution in a number of localities is quite distinct, as may be seen by comparing the geographical distribution of the two affections already outlined. It has been emphasized that in India cutaneous leishmaniasis is confined more to the western part of the country, whereas kala azar is particularly endemic in the eastern portion. Also kala azar is rare in Mesopotamia and Asia Minor where oriental sore is most frequently met with. Manson Bahr points out that in North Africa oriental sore generally occurs north of latitude 35° whereas kala azar is found south of this line.

In China in the endemic areas of kala azar cutaneous leishmaniasis does not occur or is very rare, and the same is true in the kala azar areas of the Sudan. Thus there appears to be no connection with the existence of the one disease and the prevalence of the other. According to Archibald, Turkestan, southern Italy and Sicily represent exceptions, for cutaneous lesions and kala azar in these countries are endemic in the same areas.

In the endemic areas oriental sore like kala azar seems to have a seasonal preference, making its appearance in many localities between September and January (Manson Bahr). In Turkestan Archibald says the greater number of cases occur in the months of July and August. In São Paulo, according to Da Silveira, it appears only in the late summer and autumn months, while in Rio de Janeiro Cerqueira reported an epidemic from May to August. In the forested regions of South America it prevails particularly in the rainy season. In India, however, Patton points out that it appears particularly in the provinces with a colder climate and during the cooler season of the year. In Greece Higoumenakis says it appears almost regularly after the end of summer towards the months of September and October, more rarely from November to February, while after January and February new cases are not observed. Mills and Machattie emphasize that in Bagdad both in human beings and in dogs the early lesions begin to make their appearance in September.

If one estimates the incubation period as about 2 or 3 months, the season in which the infection takes place may be estimated as in June or July, or the season in which insects (*Phlebotomus*) are often most abundant. However, the incubation period is very variable and may be from comparatively few days to a few months or even longer. In certain localities where the disease is very common, children usually acquire it at an early age, particularly when about 2 or 3 years old. Great variations, however, occur in the frequency of the disease in the endemic centers.

azar while one which recovered from kala azar was immune to both. Patton also recorded an instance of a patient who contracted kala azar after having recovered from oriental sore. If the parasite of kala azar is identical with that of oriental sore then it must in some way have been deprived of its virulence for kala azar is often a fatal disease and oriental sore is eminently benign.

In view of these facts Manson suggested that inoculation with cultures of *Leishmania tropica* might confer a protection against subsequent infection with kala azar and that this method might be used as a prophylactic. Manson Bahr suggests that this has to a certain degree proved to be true since Nicolle has reported some amount of immunity to generalized leishmaniasis in dogs and monkeys by injecting them with cultures of *Leishmania tropica*.

Wenyon points out that the distribution of kala azar and oriental sore is against the view of the identity of the two parasites, *Leishmania tropica* and *Leishmania donovani*, though undoubtedly many arguments could be raised in support of their inclusion in a single species.



FIG. 65.—Flagellate forms of *Leishmania* obtained in culture from a case of Vitis (Harvard Expedition to South America 1913)

It may sometimes be difficult to differentiate *Leishmania* from certain blastomyces. These blastomyces often stain in a manner closely resembling *Leishmania*.

The writer first pointed out this fact in 1906 in a description of the etiology of tropical ulcerations of the skin when reporting a case of human infection due to *Cryptococcus farciminosus*, the organism of lymphangitis epizootica. Rocha Lima (1912) subsequently drew attention to this source of error and pointed out that the organism described by Darling in Panama as a Protozoan under the name of *Histoplasma capsulatum* was in reality a yeast like one from a case of blastomycosis. To one familiar with both leishmania and blastomyces however there should be no difficulty in differentiation from a morphological standpoint. Furthermore cultures will at once reveal the difference since in the case of blastomycosis budding forms will be observed.

**Cultivation**—*Leishmania tropica* grows in artificial media as readily as *Leishmania donovani*. Cultures of *Leishmania tropica* are also similar to those of *Leishmania donovani* though in some instances it seems

The relationship between the human visceral and canine leishmaniasis is discussed on p 241 of this volume

**Susceptibility of Animals to *Leishmania Tropica***—Nicolle and Manceaux (1910) first showed that dogs could be inoculated successfully in the skin with *Leishmania tropica* and that local cutaneous lesions containing the parasites resulted. Dogs may be inoculated with material containing the parasites taken directly from human cases or by cultures. Laveran found that dogs which had recovered from a first infection could be reinoculated but a second attack conferred an immunity against reinfection. Mills, Machattie and Chadwick have shown that in general the histological structure of the lesions which occur in dogs are similar to those found in man.

In addition to dogs cats, monkeys, rats, mice and guinea pigs may be infected with *Leishmania tropica*. Machattie and Mills have reported 3 cases of natural infection in the cat and have also encountered it in a brown bear kept in captivity in Iraq. It has already been pointed out that in mice intraperitoneal inoculations may result in generalized infections resembling in many respects the conditions produced by the inoculation of *Leishmania donovani*.

Gupta found that when *Leishmania tropica* was injected intraperitoneally into a mouse a nodule resulted at the point of injection in addition to infection of the viscera and that when the organism of dermal leishmanoid was injected intraperitoneally a visceral infection alone was produced. However he thought that the growth of *Leishmania tropica* was far more rapid and luxuriant than that of the parasite of dermal leishmanoid.

### TRANSMISSION

**Direct Inoculation**—It has been recognized that second attacks of oriental sore are not liable to occur and particularly for this reason the Jews in Bagdad and Mosul in early years are said to have inoculated their children on the body or arms with material from a sore in order to protect them from the development of a lesion on the face with the resulting disfigurement from the scar. Some of the earlier attempts at inoculation of the disease have already been considered under History p 293. Such results were of course unconvincing since the parasite causing the disease had not been discovered.

However definite evidence of the transference of the parasite from man to man was first produced by Marzinzowsky (1909) who inoculated himself parasites being demonstrated in the lesions which first appeared 70 days after the inoculation.

Nicolle and Manceaux, Wenyon, Patton, Bonlieux, Adler and Panja have demonstrated the inoculability of cultures of *Leishmania* by infecting either themselves or human volunteers with oriental sore by this method.

Montenegro in Brazil demonstrated that the South American form was also inoculable and that the infection could be transmitted from sick individuals to healthy ones and also that the affection was auto-inoculable. However the inoculations of leishmania cultures into an already infected person resulted negatively.

While children are so commonly attacked in certain badly infected endemic areas as statistics conclusively demonstrate, it is questionable to what extent age is a predisposing factor. Canaan suggests that in Aleppo children with more delicate skins than adults are more susceptible. However, children are more likely to be exposed both to direct and indirect transmission of the infection, and after having had the disease in childhood acquire immunity to infection in later life.

Oriental sore apparently affects both sexes equally. The effect of occupation as a predisposing factor is sometimes seen particularly in the Western hemisphere where the disease is more commonly observed in those whose occupation necessitates residence in or adjacent to the country where there is dense vegetation. Hence, it is more prevalent in forest laborers, gum collectors, chicleros, and workers in yerba maté plantations. In such localities the disease prevails particularly among adult males as females are apt to be scarce in those places. In the endemic areas all races and social classes may be affected and robust individuals as well as debilitated persons may be attacked as are visitors and tourists.

At times oriental sore has appeared almost in epidemic form as in early years in Biskra and certain regions of central Asia and more recently in Paraguay among the forest laborers.

Canaan, in explaining the increased incidence of oriental sore in Palestine, considers that no cause other than the sandfly is responsible for the infection and explains the fact that in Aleppo oriental sore is much more common than in Jericho by the greater incidence of infected sandflies in the former locality. The fact that in 78.5 per cent of the cases the eruption first appeared in the summer months while in the remaining 21.5 per cent it appeared in the autumn or early winter is explained by the theory of sandfly transmission for in Jericho these flies do not occur or are very rarely seen between the end of December and the beginning of April. He observed that people who visit Jericho in the day time only do not as a rule become infected.

**Oriental Sore in Dogs**—Oriental sore as well as kala azar occurs as a natural disease in dogs. Leishmania have been demonstrated in the cutaneous lesions of dogs and in Persia also in the visceral organs. The disease has also been experimentally induced. The parasite found in canine lesions called *Leishmania canis* is however serologically identical with *Leishmania dono ani* (Chodukin and Sofieff) and the experiments of Adler and Theodor seem to prove conclusively that there is a cutaneous leishmaniasis which is common to man and dog. Mills and Machattie have pointed out that in Bagdad the human and canine diseases are commonly found to be endemic in the same areas.

The relationship of the dog to human leishmaniasis has been discussed under the transmission of kala azar. Sinton (1939) has been able to produce oriental sore in a European whom he inoculated with fluid containing *Leishmania* from an ulcer on the nose of a dog in the Punjab. Another volunteer was inoculated from this European who also developed a papule in which *Leishmania* were found.

Adler and Theodor as early as 1925 found in Palestine in *Phlebotomus papatasi* numerous *Leptomonads* in the whole extent of the alimentary canal including the oesophagus and diverticulum. The flagellates were inoculated into the skin of a human being. Thirty five days later a small papule had formed and in it *Leishmania* were found.

They also produced oriental sore by inoculating flagellates found in *Phlebotomus papatasi* into other human volunteers and observed that other flies feeding on the sores themselves derived parasites from them.

In a further study of the distribution of oriental sore, infantile kala azar and of sandflies in Palestine, Mesopotamia and Syria, Adler and Theodor found that both experimental and epidemiological evidence indicated that both *Phlebotomus papatasi* and *Phlebotomus sergenti* are the carriers of the parasites of oriental sore. In Bagdad *Phlebotomus sergenti* was found to be the main carrier. In Jerusalem and Bar Elias *Phlebotomus papatasi* appeared to be the sole carrier. In Aleppo the evidence indicated that both species transmit the disease.

From further work they concluded that *Phlebotomus papatasi* could be excluded as an important vector in Catania, Sicily, while *Phlebotomus perniciosus* which gave a higher infection rate after the ingestion of relatively few parasites, should be considered as the important carrier of kala azar in Italy. They also found that *Phlebotomus perniciosus* infected itself with *Leishmania* by ingesting skin juices of infected dogs during the act of biting. Adler and Theodor have also demonstrated the exit of *Leishmania infantum* from the proboscis of *Phlebotomus perniciosus*.

More recent work has been performed by Adler and Ber (1941) in which Sandflies (*P. papatasi*) were infected with *Leishmania tropica* by feeding through a membrane on a suspension of the flagellates in a mixture of saline and desbrinated rabbit blood. These flies were taken out from time to time and fed on susceptible volunteers to the number of 5, all of whom became infected with oriental sore. In 3 more than one sore developed.

Laveran (1880) first suggested that oriental sore might be due to fly transmission and that flies feeding upon infected dogs carried the organism on their feet and proboscides and so gave rise mechanically to infection.

It is highly probable that the house fly, which may swarm around the exposed sores, especially in children, may sometimes carry the virus on its feet or proboscis to abrasions of the skin of another person. The *Leishmania* may also pass rapidly through the gut of the fly and be deposited upon the skin with the dejecta, as sometimes occurs with certain other protozoa. Thompson and Lamborn (1934) show that transmission is particularly likely to occur mechanically by the species *Musca spectanda*. Wenyon also showed that *Stomoxys* is capable of taking up *Leishmania* from a sore, but no development of the parasite takes place.

Berberian (1938) emphasizes that while it is conceivable that infection may be accomplished by crushing an infected sandfly at the site of the bite, the likelihood of this occurring, he thinks, would appear to be rather remote. He also points out that critical experiments designed to demonstrate the transmission by the bite of the sandfly have regularly failed. He has carried out experiments especially with *Stomoxys calcitrans* (stable flies) at various times. Seven *Stomoxys calcitrans* were allowed to bite on an oriental sore and then immediately transferred to an area of skin on the thigh of the volunteer. In all 7 flies inflicted 11 bites. Five to 6 months later 2 papules appeared in the bitten area and in the first of these *Leishmania* were found. He suggests that transmission of oriental

Wenyon showed that the virus does not appear to be able to pass through the healthy skin. Material from a sore was placed on the skin and allowed to dry naturally but no lesion developed at this spot though at another spot where the skin was scarified a typical lesion resulted. This fact has been amply confirmed by many writers.

Numerous instances have been noted of individuals who have developed oriental sore at the site of some wound or abrasion of the skin and it has also been noted that a person with one sore may infect himself automatically by scratching on other portions of the body. All of these results would seem to indicate that natural infection may occur from time to time by direct contact with a person suffering from oriental sore. The occurrence of multiple cases in families which sometimes have occurred one after the other has been reported by numbers of investigators and also indicates the great probability that natural transmission of the infection may occur through direct contact.

Laveran and other authors emphasize that infection of the healthy may occur from the use of linen and other such articles which have been used by individuals suffering from oriental sore. However there is no evidence that any forms of *Leishmania* resist thorough drying.

On the other hand Parrot believes that transmission of oriental sore by direct contact is unlikely because the *Leishmania* as they exist in the infected tissues are immobile and in no way capable of penetrating the intact skin. He admits that oriental sore may develop on the site of a traumatism but says if this were a common mode of transmission the lesion would occur most frequently on that part of the body most exposed to slight traumas i.e. the hands and fingers. But in 49 cases of oriental sore observed in Algiers (Biskra) lesions were found on the hands in only 9 or less than 4 per cent. If direct contagion were the common method of spread of oriental sore it would show no seasonal incidence but at Biskra there is a definite seasonal incidence. Parrot is of the opinion that direct contagion cannot be assumed to be a common method of infection in oriental sore unless it can be shown to occur frequently in areas where the *Phlebotomus* flies are not present or are found only in insignificant numbers.

It must be admitted that oriental sore when introduced into certain localities shows no tendency whatever to spread. Whether this is dependent upon some meteorological or climatological condition or upon the absence of some transmitting insect has not been conclusively demonstrated. Thus oriental sore has been very frequently introduced into France but it shows no tendency to spread to any extent there.

**Transmission by Insects** *Sandflies* —It has long been suspected that sandflies are concerned in the spread of the disease and there is much evidence that several species of the genus *Phlebotomus* are involved in its transmission. These flies were first suggested as possible vectors by Pressat (1905) while the experiments conducted by Wenyon (1911) in Aleppo the Sergeants Parrot Donatien and Beguet (1931) in Algiers by Aragao (192 ) in Brazil Adler and Theodor (1924-193 ) in Palestine and Jerusalem and by Laveran and Franchini (1920) in France have given much support to the view that *Phlebotomus* may transmit the infection.

In South America instances are recorded of tropical sores developing at the sites of bites of *Phlebotomus*. Aragao (1922) found *Herpetomonas* in some wild *Phlebotomus intermedius* in Rio de Janeiro where local outbreaks of the disease had occurred. He fed 5 of these flies on espondia ulcerations and later found similar flagellates in them. An emulsion of the flies was then inoculated into the nose of a dog and an ulcerating lesion developed three months later in which *Leishmania* were found. Sequiera (1923) also reported 5 cases of American leishmaniasis following the bite of *Phlebotomus lut* 1 (*P. intermedius*).

hence these experiments do not give any further evidence that the tick should be regarded as the transmitting agent in nature. The mere presence of *Leishmania* is not proof of transmission. Malamos (1938) has also found in experiments performed with ticks attempting infection from hamster to hamster that all failed. *Leishmania* merely survived in the ticks in the gut for varying periods of time.

### PATHOLOGY

The gross pathology of the condition varies with the stage of the infection and the pathological histology also changes with the course of the disease. The pathological changes are obviously somewhat different in the papular and ulcerative stages. In the papular stage there is an infiltration of the corium and its papillae with plasma cells, lymphocytes and large endothelial macrophages. Frequently there is thinning and atrophy of the overlying epidermis.

The histological appearance in the early stages is that of granulation tissue. As the lesion progresses there is an increase of perivascular infiltration and polymorphonuclear leucocytes become more numerous. Most striking is the appearance sometimes observed of focal accumulations of endothelial phagocytes (clasmatocytes, histocytes or reticulo endothelial cells). These cells are frequently swollen and contain large numbers of *Leishmania*. After the lesion has become crusted and ulceration has formed upon removal of the crust usually granulations are visible in the floor of the ulcer and there is considerable inflammatory reaction at the periphery. In this stage the cellular infiltration extends deep into the corium and subcutaneous connective tissue. In addition to the endothelial phagocytes, plasma cells and lymphocytes, occasional giant cells may be seen. After ulceration polymorphonuclear leucocytes are usually very numerous. The *Leishmania* are frequently difficult to find in the ulcers and may only be encountered at the margin of the lesions or in scrapings of the floor of the ulcers.

In the later stages when the *Leishmania* have disappeared or become very scarce there is a greater increase of fibroblasts and of fibrous connective tissue with deposition of collagen fibrils as the lesion heals.

In the Mediterranean area and in Asia and Africa ulceration that results is usually comparatively superficial. *Leishmania tropica* does not commonly cause suppuration and as the writer has pointed out the micro organisms of the genus *Leishmania* cannot in any sense be termed pyogenic although they all have the power of causing extensive endothelial proliferation. Even when present in very large numbers in the liver and spleen *Leishmania donovani* does not give rise to suppuration. Only when the mucous membranes are attacked and destroyed by *Leishmania tropica* as in nasopharyngeal leishmaniasis where bacteria are almost invariably present do the lesions assume a more severe and chronic character.

In these situations it seems probable that the lesions are extended and modified particularly by the various bacteria that are present and that develop in them particularly staphylococci and bacilli. In one instance observed by the writer in Amazonia in which there was marked destruc-



sore by means of stable flies is possible and in view of the ease of transmission that this method may occur frequently under natural conditions

**Other Insects**—Have been proposed to be concerned in transmission. In earlier years bed bugs were regarded as capable of transmission of oriental sore. The evidence with reference to the bedbug in kala azar has already been discussed p 248. Blacklock and Lounie have shown that viable forms of *Leishmania tropica* may be passed in the bedbug up to 35 days in the faeces of artificially infected bugs of the species *Cimex lectularius*. Wenyon however emphasizes that no host can be regarded as being conclusively incriminated in the transmission of *Leishmania tropica* or any other parasite until the infection has been actually transmitted by it. Adler and Theodor point out that the behavior of both *Leishmania donovani* and *Leishmania tropica* in the bedbug is quite different from their respective development in *Phlebotomus argentipes* and *Phlebotomus papatasi*. In the bedbug there is no tendency for the flagellates to ascend to the cardia or pharynx.

With reference to fleas Wenyon and Laveran secured no evidence that these insects were in any way concerned in transmission. Patton also could obtain no evidence of the development of *Leishmania tropica* in *Pediculus*.

**Hippoboscidae**—Gachet observed that the dogs of Teheran were frequently infected heavily with *Hippobosca canina*. In examining a fly which had just fed upon a sore on the face of a dog *Leishmania* were found in the blood in its stomach. Gachet has suggested that the frequency of cutaneous leishmaniasis of dogs in Teheran may be due to the prevalence of this fly.

This is of some interest in connection with the transmission of cutaneous leishmaniasis (bay sore) in Yucatan especially among the chiclero workers. Shattuck and Bequaert point out that it can hardly be doubted that some biting insect is the transmitter but that no experiments have yet been published showing which particular species is involved. There is much local opinion in Yucatan and northern Guatemala incriminating the fly of the ocellated turkey or of the bare throated guan.

Farfan has suggested that *Olfersia coriacea* may be concerned in the transmission and it has been stated that leishmaniasis has been transmitted through its bite. Shattuck and Bequaert however point out that it seems more likely that in Yucatan as elsewhere in the New World, cutaneous leishmaniasis is transmitted by one or more species of *Phlebotomus*. Bequaert also says that Hippoboscidae as a rule are not prone to bite man after leaving their normal host though there is certain evidence to the effect that some European Hippoboscidae will bite human beings.

Van Thiel has called attention to the possibility of the transmission of American leishmaniasis by the patatta mite *Trombicula flus*. In South America the transmission of the infection by ticks has long been suspected. Although Weiss, Escomel and Ribeyro and Bambaren have debated the possibility van Thiel after considering all the evidence states that he does not believe that the patatta mite is concerned in the transmission of leishmaniasis in Surinam.

With reference to the transmission of American leishmaniasis Brumpt thinks the fact that dogs are susceptible as well as monkeys to inoculation with the parasite and are often bitten by ticks without the production of the sores is against the view that ticks act as the transmitting agent.

In the discussion of the transmission of visceral leishmaniasis it was noted that *Leishmania* will survive for about two weeks in larvae and nymphs of *Rhipicephalus sanguineus*. However they will even survive for longer periods in the bed bug and

may undergo fibrosis. In the late stages the accompanying endarteritis of the neighboring vessels leads to extensive ulceration and necrosis. The *Leishmania* when present in the lesions were almost constantly situated intracellularly within endothelial cells. They believe that the continuous spread of the lesions throughout the nose and to the pharynx was in all probability related to the lymphatic drainage of these parts and that the lymphatic channels play an important role in the local spread of the disease. They add however that the manner of development of the lesion of the nose suggests a metastatic distribution from a primary ulcer on the arms or legs.

Buss suggests that parasitic foci originate from the blood vessels which are of importance from the point of view of the character and course of the disease and may account for the invasion of the mucosae. The formation of tuberculoid tissue was seldom seen by him and he regarded it as a less important feature than in ordinary cases of oriental sore. In the late stages of the American cases disease tissue resembling tubercles sometimes occurred while in about one third of the cases giant cells were found. However *Leishmania* were not found in a number of the cases he reports or in 3 cases of the disease studied by Fox.

Portugal in the study of Brazilian cases emphasized that the exact histological picture changes with the course of the disease and subdivides his cases into the ulcerating and non ulcerating ones. There is usually no marked resemblance between the lesions of the nasopharyngeal cases and those of lupus vulgaris.

The lymphatics and glands draining the infected regions however not uncommonly show inflammatory changes in cases of leishmaniasis.

**Immunity**—The natural infection of man with oriental sore produces a rather lasting immunity and the fact that one attack usually protects has led parents in certain localities as Bagdad to inoculate their children on the extremities to protect them from the scars and disfigurement of sores which might develop on the face. Laveran as noted found that dogs which had recovered from a first infection were reinoculable but a second attack conferred an immunity against further infection. Moses with a culture of *Leishmania* employed the complement fixation test for the diagnosis of cases of South American leishmaniasis and believed that an immune reaction could be obtained in 80 per cent of the cases.

Waggener, Jessner, Amster, Montenegro, Buss and daCunha have also studied the cutaneous reaction as a means of diagnosis and believe that it may be of value in a large proportion of the cases.

Foot in referring to the fact that one attack of cutaneous leishmaniasis confers an immunity that is usually lasting has considered the proposal of vaccinating against this disease. However in employing an antigen from infected tissues he was not successful in protecting monkeys from infection.

Kurotchkin in attempting to immunize hamsters against *Leishmania donovani* found that vaccines prepared from flagellates and non flagellate forms of *Leishmania* did not protect the animal against infection but tended to render them even more susceptible to inoculation. He believes susceptibility of hamsters was absolute and the infection might occur even when very few parasites were injected.

tion about the nares *Bacillus mucosus capsulatus* was present Klotz and Lindenberg who studied 15 cases of leishmaniasis of the nose in Sao Paulo also remark that the tissue reaction due to the parasite never causes a purulent inflammation such as occurs with *Blastomyces* and *Actinomyces* Fox has also noted the occurrence of other organisms in symbiosis with *Leishmania* in certain cases

The presence of diplococci in oriental sore was first pointed out by Riehl in 1886 and in the same year Loustalot and Leloir cultivated a micro-organism which they thought at first was specific Subsequently numerous other investigators reported cocci as of significance in connection with the etiology or as complicating the disease

Arias and Rosa state that by means of roentgen ray photographs they have been able to demonstrate that cutaneous ulcers due to *Leishmania* are liable to produce a condition of osteitis in the bones which lie beneath them They do not however emphasize the question of secondary infection with other micro organisms than *Leishmania* in such pathological changes of the bone Mazza and Cornejo (1935) have also observed two other such cases with osteitis of the metacarpal bones beneath the lesion

Ulcerations of the mucous membranes show histologically complete disappearance of the epithelial layer often with coagulation necrosis of the surface tissue There is usually a fibrinous leucocytic exudate forming a false membrane beneath which there is a dense cellular infiltration in which large endothelial phagocytes polymorphonuclear leucocytes plasma cells and fibroblasts are found lying in a vascular stroma of fine or coarse connective tissue The lymphatics and capillaries are often dilated the latter sometimes being occluded by red cells The *Leishmania* may sometimes be found present at the periphery of the lesions within endothelial phagocytes However in cases of long standing where secondary infections with bacteria have occurred the *Leishmania* are very scarce and may be entirely absent The blood vessels in uncomplicated cases usually show no distinct evidence of endarteritis In general, in the late lesions also one finds a picture of a chronic inflammatory process with the production of ordinary granulation tissue in which there is more or less cellular infiltration and in which occasional giant cells are present In the keloid type of leishmaniasis noted in the Sudan by Balfour and Thomson epithelial cell nests were described Archibald also noted thickening of the epidermis with the formation of epithelial downgrowth although there were no other evidences of epithelioma

Klotz and Lindenberg Llambias and Mosto Buss Portugal and Fox and Highman have made pathological studies of South American cutaneous leishmaniasis Klotz and Lindenberg describe the pathological histology of the lesions of the nose and find that the granuloma begins as a perivascular lymphocytic infiltration of the submucosa which passes through stages in which the plasma cells and endothelial cells gradually gain dominance and ends with the development of nodules largely composed of endothelial cells These nodules may show central necrosis or

with *Spirochaeta*. The sores which are usually in the neighborhood of 2.5 cm. in diameter may in some instances come to occupy an area 6 to 8 cm. in diameter. In the great majority of cases which are treated the ulcerations do not penetrate to great depth and when the infective agent is destroyed the ulcer heals by granulation and a scar results.

The lesions are generally confined to the exposed surfaces—hands, feet, arms, legs, and especially in young children on the face, rarely on the trunk, never on the palms, soles of the feet, or hairy scalp. Frequently in Yucatan and in some cases in Paraguay they have occurred about the ears. Higoumenakis, in stating that the lesions occur almost exclusively upon the uncovered parts, cites 2 cases reported by Loghan in Teheran in which they were situated upon the hypogastrum. These 2 cases were in women and were explained by the fact that it is a custom for the natives in that locality to keep the hypogastrum uncovered.

Weber, in a study of the distribution of the lesions, found about 85 per cent of the sores located on the upper or lower extremities and about 10 per cent on the face, while the trunk served as the location in only about 5 per cent of the cases. Fox, in the observation of 50 cases in São Paulo, found the mucous membranes involved in 10 cases, 20 per cent. The location of the disease in 4 was the nose, cheeks, or lips. One case presented a single lesion of the ear, and 2 were noted on the chin, and 2 on the shoulder. The majority of the lesions were on the extremities, especially the lower, at points distal to the knees and elbows.

Lesions are often single, but 2 or 3 sores are not uncommon. Manson Bahr states that in many instances as many as 150 have been counted upon the same patient. Cardamatis and Melissidis (1911) recorded a case in Greece in which there were 35 sores distributed about the hands, arms, and face. Torres reported one in South America in which 248 distinct lesions occurred on various parts of the body, while Buss has reported and illustrated a case in which there were over 300 nodular lesions, papular, crusty efflorescences of three months' duration. Deprete and Beinert found the number of sores in a case was one in about 30 per cent, 2 to 4 in 50 per cent, and from 4 to 20 in about 20 per cent of the cases. In 50 cases in São Paulo, Fox found the number of lesions for each case averaged 3.3.

As a rule there are no constitutional disturbances observed except in some of the South American cases in which extensive nasopharyngeal involvement exists and the patient is sometimes greatly reduced in health. In such cases the lymphatic glands and lymphatics draining the infected region are involved and in some instances the glands have been found to contain *Leishmania*.

**Blood.**—In cutaneous leishmaniasis there is usually no diminution in the number of red blood cells. A slight increase of the large mononuclear leucocytes has been noted in some instances. Archibald states that this increase is more marked in the blood taken from or close to the area where the lesion exists. In cases of nonulcerative cutaneous leishmaniasis the Sudan blood has usually shown a leukopenia and an eosinophilia. Higoumenakis (1930) who examined both the blood from the finger near the oriental sore as well as the peripheral blood, found that the blood taken near the sore usually showed a great increase in the medium-sized mononuclear cells.

## SYMPTOMATOLOGY

**Incubation Period**—The incubation period of oriental sore has been stated to vary from a few weeks to many months. Manson Bahr states that it may be even briefer, the sore appearing sometimes even a few days or weeks after the arrival of the individual in endemic districts or after inoculation. On the other hand, that it can be of much longer duration is equally certain. Manson reported an unquestionable oriental sore which did not appear until 5 months after the patient had been exposed to any possibility of infection.

Marzinowsky, who inoculated himself, developed a lesion 70 days after the inoculation, while in the case of Wenyon, who also inoculated



FIG. 66—Oriental sore (After Cardamatis)

himself in Aleppo, it was not until nearly 7 months later that a minute lesion appeared and only subsequently did ulceration develop.

**Cutaneous Lesions**—The disease begins with a small insignificant appearing papule which is in some instances attributed to an insect bite. The papule gradually increases in size and after a month or two a lesion measuring frequently from 1 to 3 cm. in diameter may be formed. In some instances, after persisting for 6 months to a year, the lesion apparently atrophies and becomes dried and covered with a superficial scab or scaling surface which eventually falls off, leaving a depressed scar. However, in the majority of cases, the lesion becomes covered by a more or less dark crust from which a sticky secretion exudes. On the removal of the crust, a moist superficial ulcer which bleeds freely is revealed. In neglected cases, the ulcerations extend slowly in size and depth, and the lesion usually becomes secondarily infected with bacteria and sometimes

In the cases simulating verrucous tuberculosis it is necessary to demonstrate *Leishmania* and the absence of the tubercle bacillus before establishing the diagnosis.

The writer also observed cutaneous lesions of the left thigh and leg in a woman in Manaos, Amazonia. The lesions were said to have had a duration of 7 months.

Christopherson has described discrete lupus like nodules occurring on the cheek, in general appearance resembling lupus vulgaris but containing *Leishmania*. In the South American cases which involve the nasopharyngeal areas there is usually no marked resemblance between the leishmania lesions and those of lupus. However, Sinderson (1931) has



FIG. 68.—Lesion of the type I of chiklero ulcer. (From case of Dr. G. C. Shattuck, Courtesy Carnegie Institution of Washington.)

called attention to the occurrence of a secondary tuberculide in oriental sore in children in Bagdad and suggests that the leishmania infection may predispose to the development of the lupus.

**Chiklero Ulcer**—The disease in Yucatan called chiklero ulcer has been recently investigated carefully by Shattuck, who says that it seems to have clinical features which separate it in a measure from the forms observed in other parts of the world.

The disease affects particularly the native chiklero workers, though the pure Mayas even when they live in the forest seem seldom to be attacked by it. It occurs in either sex but is much more common in the males in the forest camps but as Shattuck points out this seems unimportant as comparatively few Mestizo or Mexican females live in these camps. In males it was encountered between the ages of 9-28 years and in females from 9-16 years.

Mazza and Niro who examined 65 cases of South American disease in Brazil found a definite increase in the mononuclear cells. In the peripheral blood there was an average mononuclear count of 41 per cent and of these 36 per cent were lymphocytes. In the blood taken from the region of the sore the figures were 48 mononuclears and 45 lymphocytes. In the peripheral blood there was an average of 6 per cent eosinophils.

In cases of oriental sore the *Leishmania* do not occur as a general rule in the peripheral blood.

Both Wenyon and Archibald who searched for them on many occasions and Mazza who recently sought for them in 39 cases of the South American disease had only negative results. From 35 of these cases cultures were made from the peripheral blood but these likewise were negative. In the majority of the cases parasites were found in the material taken from the lesions. As great exceptions Neumann on a few occasions and Patton on 1 have however reported their presence in the blood.



FIG. 67.—Skin involvement in Brazilian leishmaniasis (After da Silva.)

With reference to the Wassermann reaction Bernasconi tested this according to the technique of Wassermann Hecht and Meinicke in 35 cases of cutaneous leishmaniasis in the Argentine diagnosed by the discovery of the parasites. In 11 cases the reaction was positive with the three techniques and in 18 it was negative. In the other cases one or the other of the different techniques employed gave a negative or doubtful result. Mazza (1929) in a test of his cases found that the Wassermann reaction was negative except on rare occasions. He also found that the formal gel test and the serum water test which gave positive results in kala azar were negative.

#### Other Clinical Forms of the Disease—

Balfour and Thomson noted in the Sudan subcutaneous slow developing nodules simulating keloid growths. Material obtained from the lesions showed a heavy infection with *Leishmania*. Somewhat similar lesions were later described in India by Brahmachari and by Napier and others under the term of dermal leishmanoid. The Indian writers have regarded this lesion as a post kala azar manifestation.

It has already been described in detail under the visceral form of the disease.

Ferguson and Richards have described in Egypt a verrucous form while Higoumenakis has referred to a form simulating verrucous tuberculosis. The lesions described by Ferguson and Richards affected particularly the lower extremities and resembled warty outgrowths of papules. They were solitary or multiple and could be the result of auto inoculation.

there has not been the extensive involvement of the postpharyngeal region sometimes encountered in the disease in South America. Rabello states that in Brazil 90 per cent of the cases resemble oriental sore and that secondary infection occurs in the remainder so he does not think the disease in Brazil is a separate one. Nevertheless the prevalence of the lesions of the mucosa in the South American cases has led many writers to classify and describe such lesions under the term American leishmaniasis and the writer has not observed such lesions due to *Leishmania* in any other parts of the tropical world.

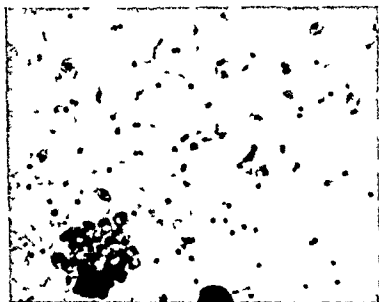


FIG. 69.—Film from scrapings from the buccopharyngeal cavity containing Leishman-Donovan bodies lying free in the field (Case of Dr. G. G. Shattuck, City College of Medicine, Washington).

The percentage of cases with mucous lesions varies in the different localities. Da Silva reported involvement of the mucous membranes of the nose, throat and mouth in about 20 per cent of 15,000 cases in São Paulo. The most heavily infected endemic areas are in Brazil, Paraguay and Peru where the disease has occurred particularly in men working in forests or districts where there is luxuriant tropical vegetation. On the other hand, in certain localities in Peru cases occur in the mountainous districts. In Paraguay it has been especially observed in those working in the forests and maté plantations in which it is said to have assumed almost epidemic form, 70 to 80 per cent of the laborers being infected.

In British, French and Dutch Guiana it is also seen most commonly in the forest laborers and the same is true in Yucatan among the chicleros.



The early lesions consisted of dark red papules a few millimeters in diameter. In the second stage the papule has a diameter of from 1 to 2 cm. is markedly elevated, and is surrounded by a zone of inflammatory oedema. The papule however does not resemble a boil. It develops slowly, is cyanotic rather than red in color, does not contain a pocket of pus and is soon covered by a scab composed of dried serum and blood under which there is a small ulcer. The third stage is characterized by the accumulation of pus beneath the scab, by loosening of the scab and finally by loss of the scab. Thus is exposed a shallow ulcer buried in pus. The edges of the ulcers are definitely elevated and not undermined, but they may be relatively smooth or ragged. The ulcer may increase in extent and in depth by sloughing. When it develops upon the ear it may result in the loss of a considerable portion of that member by sloughing (Fig. 68). When the fourth stage the healing process has begun the ulcer can readily be washed clean and the base is then composed of red healthy looking granulation tissue.

During the sloughing stage there is often much pain. Shattuck points out that his experience, as well as that of others, indicates that the initial lesion in the great majority of the cases appears on the ear and that usually there is but one lesion. On the other hand both ears may be attacked and the initial lesion may appear on the arm or else where. The youngest lesion which he saw was situated upon the hand, but in this case the patient had 16 lesions in various stages of development. The disease had begun 3 months before and apparently the lesions had been increased in number by auto-inoculation into the abrasions of the skin, probably as a result of scratching. The early lesion on the hand was the last to develop and was papular in character.

Shattuck confirmed Seidelin's suggestion that the disease might be a form of leishmaniasis and he demonstrated *Leishmania tropica* in the lesions. He emphasizes the importance of finding *Leishmania* before diagnosis is made and points out that if this is not done errors in diagnosis may occur. However in some of the cases which seemed typical *Leishmania* were not demonstrated. Shattuck says that he has no doubt that secondary bacterial infection plays an important role by aggravating the lesions and that most of the pus and sloughs result from the action of bacteria. He says that while there are many reasons for believing that the disease is transmitted by an insect it seems to him highly improbable that a hypoboscoid of birds is the agent of transmission and that it seems highly probable that this and other forms of leishmaniasis are transmitted usually by certain species of *Phlebotomus*.

**American Leishmaniasis**—While in most cases of oriental sore in South America, as well as elsewhere in the endemic regions of the world, the lesions are confined to the skin, in about 10 to 20 per cent of the South American cases the mucosae are involved. In such cases the ulcerations and necrosis in the nose, mouth, and pharynx may result in most extensive and distressing lesions and lead to a profound cachectic condition of the patient. However ulcerating lesions of the mucosae not only occur in South America, but occasionally also in the endemic regions elsewhere in the world. Cases in which ulcerations may extend from the skin to the inner surface of the lips and nose have been recorded by Cardamatis and Melissidas in Greece, by Pulvirenti La Cava in Italy, and by Christopherson in the Sudan. Eller also records 2 cases in Madrid with lesions of the mucous membranes of the mouth. In most of these cases, however

Escomel differentiated uta as a form of the disease in which the skin is the part affected and in which the process extends peripherally from the primary lesion and without any breach of continuity. On the other hand in espundia the disease was said to first attack the skin and then spread to the mucous membranes so that there is an interruption of continuity between the initial lesion and the secondary or tertiary ones. After many months the initial sore is said to heal, nodules and ulcers appear then or later in the nasopharynx. Such lesions progress slowly with severe destruction, mutilation and scarring until death occurs from sepsis and malnutrition due to interference with deglutition unless treatment is instituted. The secondary lesions in espundia it is said occur most frequently on the mucous membranes of the upper respiratory and digestive tracts extending from the nares to the trachea and sometimes from the lips to the oesophagus. In some cases of palatal ulcer two deep furrows are produced, one along the edge of the soft palate and the other crosswise in the median palatal line forming a sign which is known as the palatal cross of espundia.

### DIAGNOSIS, PROGNOSIS AND PROPHYLAXIS

**Diagnosis**—Without the finding of *Leishmania* in the lesions certain diagnosis is impossible. In instances in which the parasites cannot be demonstrated microscopically either in scrapings from the edge of the lesions or in a drop of serum withdrawn by syringe needle or pipette from the periphery they may sometimes be obtained by culture inoculated with the serum thus collected.

Wenyon relates a case in which though scrapings from the sore and puncture of the margin failed to reveal *Leishmania* in stained films yet the flagellates grew in cultures inoculated with material obtained by puncture after sterilization of the skin. The organisms must have been very scanty for it was not until after the lapse of three weeks that the parasites had multiplied sufficiently to be detected. However in some cases when the parasites have been observed by microscopical examination the cultures have been negative. This is likely to be the case when bacteria predominate in the lesion as in the ulcerative cases. For this reason Wenyon and Connor and Shortt emphasize that the material for cultures should be obtained by inserting a fine drawn pipette run beneath the surrounding skin which has previously been disinfected. However as such a pipette is likely to break in inserting it through the skin a hypodermic syringe often gives better results. Connor and Shortt emphasize that the parasites are by no means always easy to find in the sores particularly on account of secondary infection. Of 187 consecutive suspected cases parasites were demonstrated in only 95. One obviously could not conclude that the remaining cases were definitely leishmaniasis.

The dermal reaction has been employed in human cases but further study as to the value of this test as a means of diagnosis is necessary.

**Differential Diagnosis**—From clinical appearances oriental sore may sometimes be confused with blastomycosis and possibly with lesions of tertiary syphilis, leprosy and phagedaenic ulcer while the advanced nasopharyngeal lesions may be confused with facial lupus, nasal syphilis, gangosa, glanders, rhinoscleroma, nasal myiasis, leprosy and venereal granuloma. In such lesions unless *Leishmania* have been demonstrated either early in the disease or in the late lesions a definite diagnosis of

workers In Yucatan the lesions have been found especially about the ears However in these localities involvement of the mucous membranes is not a prominent feature

In Peru the disease has been known especially under 2 names *esputa* and *uta* but in Brazil with the exception of parts of Amazonia these terms are not used instead the affection is sometimes termed

Bubos Tyzzer Sellards Brues Gastiaburu and the writer who studied *uta* in Peru in earlier years found that the disease began as is



FIG 70—Skin and mucous membrane involvement in Brazilian leishmaniasis (Oswaldo Cruz Institute)

usual with oriental sore with a small papule which soon increased in size and became covered by more or less moist dark crusts from which a sticky secretion exuded On removal of the crust a moist ulcer was revealed which bled freely These ulcerations in neglected cases extended slowly in size and in depth when the lesions became secondarily infected with various micro-organisms Eventually the soft and hard palate sometimes became destroyed by the extension of the lesion and in some cases the walls of the pharynx were eaten away When the larvae of *Chrysomya macellaria* penetrate into the depths of the lesions the ulcerations may become very extensive and serious

for the exclusion of *Phlebotomus* and the employment of insect repellants is recommended. Individuals with oriental sore should be informed of the danger of their infecting others by personal contact as well as of auto-infection through scratching. The lesions should be protected by dressings to prevent the access of flies and other insects which might convey the infection either as an intermediate host or mechanically. All linen or dressings which have come into contact with the sores should be destroyed by burning.

All cases of the disease should be treated. In badly infected regions the systematic treatment of the cases is obviously an important prophylactic measure. In regions where natural canine infection occurs the dogs and especially infected dogs should be destroyed.

### TREATMENT

**Antimony Preparations**—When the lesions are extensive or multiple the treatment usually recommended is by intravenous injections of tartar emetic or of sodium antimony tartrate as was first introduced by Vianna in 1913. A 2-4 per cent solution may be injected beginning with 0.5 gr (0.33 gm) doses. The solution of antimony should be made up freshly. Injections may be given every third day. In some cases 10-15 gr (6-1 gm) are sufficient to effect a cure. Other cases may require a longer course of treatment though 10-20 injections amounting to 20-30 gr usually suffice. Such treatment however is not invariably successful.

The employment of the pentavalent antimony compounds has also given good results and a cure has sometimes been obtained within a shorter period. With neostibosan the dose should begin with 0.1 gm and increased on alternate days to 0.3-0.4 gm. (See p. 285 for a description of these compounds.) Neoantimosan (fouadin) a trivalent antimony compound has also been used successfully in intramuscular injections.

Rogers cleared up a case of multiple oriental sore by daily injections of neostibosan in 10 days. While the usual oriental sore unless the mucous membranes are attacked heals of itself in the course of from 6-18 months by the use of intravenous injections of antimony the sore usually not only heals in a much shorter time but is apt to result in less scarring than if it is allowed to run its natural course. However on account of the more or less benign nature of many oriental sores and the toxicity of the antimony compounds many clinicians prefer to use other methods of treatment.

**Tartar emetic ointment** in the strength of 1-2 per cent for local use has been recommended by Low, Manson Bahr and Wenyon. However it is very irritating to the unbroken skin and some people cannot stand this strength. The addition of cocain 3 gr to the ounce is recommended for deadening the pain. The ointment should be smeared on the sore at night and allowed to soak in. Kennedy obtained satisfactory results with this ointment but thinks in order to do this it is necessary to produce a considerable local reaction with some sloughing and pain and he does not recommend its use for the face.

leishmaniasis is not possible. In South America undoubtedly many cases with ulcerative destructive lesions of the skin and mucous membranes have been attributed to *Leishmania* although they had a different etiology. Syphilitic lesions are particularly likely to be confused. The Wassermann reaction should be employed as an aid in the differentiation in all ulcerative cases in which leishmaniasis is suggested.

Muir, who has had a very extended experience with leprosy in India, has called attention to the confusion that has sometimes arisen in India in the diagnosis of cases of dermal leishmanoid as leprosy. In all doubtful cases leprosy bacilli should be carefully sought for in the lesions.

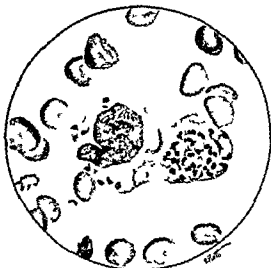


FIG. 71.—Preparation made from a leishmanoid in the early stage of Uta showing *Leishmania tropica* (*Leishmania*) the etiologic factor. (Harvard South American Expedition 1913.)

**Prognosis.**—Cutaneous leishmaniasis unless the mucous membranes are involved is not a fatal disease. If untreated however the lesions may persist for a year or longer and successive lesions may follow one another particularly through auto inoculation sometimes by scratching. When the lesions occur on the face there may be disfigurement from the contraction of the scars. Archibald has noted that when the lesion is in the region of the eyes ectropion and occlusion of the lacrimal ducts may result. Costa has also reported lesions of the lids and changes in the cornea. When the mucous membranes are extensively involved as in the cases termed espundia and uta the prognosis is sometimes unfavorable. Such lesions are usually progressive unless carefully treated. Some of these advanced cases do not respond to any treatment and the disease may end in death due to respiratory complications or exhaustion from cachexia.

**Prophylaxis.**—In areas where the disease is endemic precaution should be taken against the bites of insects. The use of special mosquito nets

ulcer is first cleaned and then covered with a pad consisting of 16 layers of lint soaked in 2 per cent zinc sulphate solution. This is firmly applied under a zinc electrode by means of a bandage and then connected with a positive pole of an electric current which is applied by 18 accumulators giving an average of 36 to 38 volts. Manson Bahr states that a patient with a sore of an area 1 inch in diameter can easily stand 10 ma. as gauged by a resistance coil. The application is continued for 20 minutes the pad being constantly moistened with zinc sulphate solution.

**Diathermy**—Higoumenakis who has written a monograph upon the subject of *Le Bouton d'Orient* has devoted much attention to the success he has had in the treatment of the affection by diathermy or bipolar application of high frequency current. Diathermocoagulation.

However Lanowsky has after the treatment by diathermy of 214 cases of oriental boil with 536 lesions arrived at a much more conservative opinion. He believes it is only possible to recommend the use of the method in the papillary forms of oriental sore. In the ulcerative forms in the treatment with diathermy only a slow healing was obtained and an unfavorable cosmetic effect on account of the formation of keloid scars while in 11 per cent of the cases there was a relapse of the condition.

Also Rotenberg and Baranowsky who have used the method in 36 cases of dermal leishmaniasis in which there were 103 oriental boils say little that is favorable about the method. Only in 16 patients in which a cure was obtained did relatively flat atrophic scars result while in all the other cases the end result was considered to be unfavorable on account of the formation either of keloids or deeply produced scars.

**Other Treatment**—Whatever the method employed the ulcerative lesions should be treated on general lines by first cleansing with some mild antiseptic solution and after the application of the medicament the lesions should be covered with a dressing. The local application of powdered permanganate of potash has been particularly recommended for the ulcerative lesions. However while it sometimes gives good results it is very painful.

A number of observers have employed an ointment of equal parts of methylene blue vaseline and lanolin (Wenyon Archibald Kardoumatis and Melissides) and preferred this to the 1 or 2 per cent antimony ointment.

Cures have been effected by scraping excision actual cautery and the use of stronger disinfectants such as carbolic and nitric acids which destroy not only the parasites with which they come in contact but unfortunately the tissues as well. Excision does not always result in a cure as a new lesion may appear outside the excised area or in another part of the body.

Among other drugs tried has been Bayer 205. Moschkowsky used it in a number of cases without apparently any beneficial results. Stovarsal was employed by Mazza and Bernasconi in 3 cases of espundia which

*Carbon Dioxide Snow*—In parts of India the application of carbon dioxide snow is regarded as the most satisfactory form of treatment. It is applied for from 5 to 30 seconds according to the size of the lesion, and repeated every 10 days. Warma has found this to be the method of choice in the Mayo Hospital at Lahore the intravenous injections of tartar emetic solution being employed more especially for cases showing multiple sores of the body.

*Emetin*—Photinos has recommended injections of emetin hydrochloride which are made beneath and around the edges of the sore. Caliceti, Panayotatou and Sinderson have also reported good results in treatment by this method. Sinderson has used either a 2 or a 5 per cent solution injecting it into the thickened edges and bases of the ulcers and using not more than 0 minims at 1 time, which was distributed over several sores if the lesions were multiple. After 3 or 4 days the sores became well defined ulcers and healed in about a fortnight.

*Berberine Sulphate*—Gupta and Dikshit and Karamchandani recommend the use of berberine sulphate. Devi has also employed this method of treatment (2 per cent solution) with 18 sores and found that 6 healed completely after 1 injection, 5 after 2 and 5 after 3 injections. In 1 case there was marked improvement after 1 injection but the patient did not return for further treatment. Only 1 case showed no improvement and in this case the diagnosis was doubtful.

DeCastro believes that a 2 per cent solution of berberine sulphate frequently causes severe burning sensation for many hours, and prefers 3 cc. of a 1 per cent solution. Napier (1931) points out that about 3 cc. is the maximum amount of the drug which it is safe to administer as the preparation is toxic. Others recommend 2 cc. of a 1 per cent solution.

*Roentgen Rays*—Treatment by roentgen ray has also been used with success in Mesopotamia and elsewhere, particularly by Tomkinson, Host, Dore and Atkins. Atkins considers that combined with zinc ionization the best results are obtained, while Triston recommends roentgen rays with applications of permanganate locally. Manson Bahr says that when roentgen rays are available this line of treatment appears to be at once rapid and efficacious and in Mesopotamia Mitchell found that a single full pastille dose of roentgen rays produced a cure within 10 days in the majority of cases. The treatment was not followed by any constitutional disturbances and the scars which were left were hardly noticeable. It is stated that the rays act directly upon the parasites.

Dostrowsky also believes that the best method is by treatment with roentgen rays pointing out that the treatment is rapid and there is less waste of time and for the patient it is painless. However, the method is expensive and not always available. Of 30 cases treated with roentgen rays only 1 was refractory. The treatment lasted from 30 to 120 days in applications with a week's interval.

*Treatment by Ionization*—Manson Bahr and Archibald described treatment by ionization which has been found to give good results. The

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resisted treatment with tartar emetic. Definite improvement was obtained after 13 or 14 injections.

Double iodide of quinine and bismuth in intramuscular injections was recommended by Vigne and Pringault and a few cases of successful treatment with it have been reported by Escomel, Agronick, Komarow and Aranda. It apparently has had no wide use since. The dose recommended is 0.15 gm. daily for 1 month.

**Gold Treatment**—Sorinsson has recommended injections of gold preparations (krysolgan and sanocrysin) and reported favorable results in 5 cases after 5 to 6 injections.

**Treatment of Mucous Lesions**—The treatment of extensive lesions of the mucosae is often difficult. It is recommended that the local ulcers on the lips and nose should be cleaned up with fomentations, the cleansed surfaces anaesthetized with a mixture of cocaine, menthol and carbolic acid and then sprinkled with finely powdered antimony tartrate and bound up with a bandage. Subsequently the wounds are dressed with an ointment composed of zinc oxide, bismuth and lanolin. In the case of lesions of the buccal mucosa the scabs may be removed with solutions of bicarbonate of soda, the surface anaesthetized with cocaine (1 per cent), and sprayed with 1 to 2 per cent antimony tartrate solution. Every 4 to 8 days the tartrate is used in a saturated solution, the application being made by means of pledgets of cotton wool.

In some instances the lesions of the mucous membranes have been found in South America not to yield satisfactorily to treatment with intravenous injections of antimony and potassium tartrate. Good results in some cases of this nature have been recorded from intramuscular injections of dioxy diamino arsenobenzol or neosalvarsan.

Andrews has recommended for the nasal manifestations in South America alkaline washes to soften and dislodge the mucus and crusts followed by applications of caustics such as trichloroacetic acid or phenol. Local treatment should be accompanied by intravenous injections such as tartar emetic.

Shattuck in the treatment of chancro ulcer states that his experience indicates that an important part of the treatment should consist in cleansing the ulcer to get rid of the bacterial infection and that an ointment such as sulphur ointment should be applied which will prevent dryness and prevent scabs from sticking to the surface. In addition injections of potassium antimony tartrate may be given.

**Treatment of Post kala azar Dermal Leishmaniasis**—Neostibosan has proved to be more effective than sodium antimony tartrate.

With reference to the treatment of oriental sore as well as of all forms of leishmaniasis it should be borne in mind that while it has been shown that a number of the pentavalent compounds of antimony are considerably less toxic to human beings and laboratory animals than the sodium and potassium salts of antimony, it has not been conclusively demonstrated that in the destruction of the parasites they are equally effective in the same dosage as the potassium and sodium salts. (See Solustibosan p. 286.)

## Chapter VII

### THE TROPICAL RELAPSING FEVERS

**Synonyms**—Febris recurrens spirochaetosis spirillum fever famine fever Carapata disease Kimputu tick fever French—Typhus recurrens German—Rückfallfieber

**Introduction**—In tropical countries a group of fevers more or less identical clinically with European relapsing fever are also caused by spirochaetes of the genus *Spirochaeta* (*Borrelia*). The first to engage the attention of physicians was the organism of European relapsing fever known for a long time as *Spirillum obermeieri* (*Spirochaeta recurrentis*) transmitted by the louse *Pediculus humanus*. The relapsing fever of Central Africa is caused by a species *Spirochaeta duttoni* which is transmitted by a tick *Ornithodoros moubata* while that of Northern Africa is caused by *S. berbera* which is transmitted by lice. A species of spirochaete named *S. carteri* causes the relapsing fever of India. It seems evident that its transmission is also brought about by infected lice as is the form encountered in China. A number of others have been reported among which are *S. norys* for American *S. persicum* for Persian relapsing fever and *S. venezuelensis* (*neotropicalis*) for the form seen in Venezuela and Panama. The view taken by Nuttall that the various names given for the different strains may be of convenience in the study of relapsing fevers but that there is no adequate morphological difference to justify them as distinct species seems worthy of acceptance. Also it has been shown that the separation of these and other named spirochaetes of the genus on the basis of susceptibility of laboratory animals and cross immunity reactions is untenable. While all the louse borne strains seem indistinguishable by agglutination methods some workers have found that tick borne strains (*S. duttoni*) are not agglutinated or lysed by a serum prepared with the louse borne strain *S. recurrentis*. Also the writer has shown that rats thoroughly immunized against *S. recurrentis* by repeated injections may still be infected with *S. duttoni*.

#### STUDY AND IDENTIFICATION OF SPIROCHAETES

##### Croup Spirochaetacea Fantham 1908 (The Spirochaetes)

**Classification**—It is still disputed whether the spirochaetes should be considered as protozoa as suggested by Schaudinn in 1905 or whether they are more closely related to the bacteria as was originally believed. At present the latter view is more generally held and Bergey in his latest *Manual of Determinative Bacteriology* 1939 places them in the class *Schizomycetes* order *Spirochaetales*.

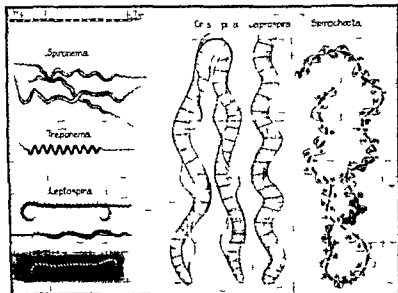
The term *Spirillum* was the generic name first applied by Ehrenberg in 1833 to large free-living spiral organisms the protoplasm of which appeared to be wound around

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obtuse ends cylindrical and composed of 2 to 5 large irregular flexures) Has a distinct and flexible longitudinal crest and an internal chambered structure like *Saprosylla*

4 *Borrelia* Swellengrebel 1907 Type *B. gillmorum* Various sizes no axial filament no crista or undulating membrane All disintegrated by 10 per cent saponin and bile salts 5 to 14  $\mu$  in length by 0.5  $\mu$  in width flexible and snakelike In man *B. recurrentis* *B. novyi* *B. duttoni* *B. hermsdorni* *B. castris* *B. rickettsii* *B. buccalis* *B. egypti* *B. bronchialis* etc Includes *B. anserina* of geese *B. tholozani* and many others

5 *Treponema* type *T. pallidum* Schaudinn 1905 Shaped like a corkscrew pointed ends 8 to 14  $\times$  0.3  $\mu$  with from 6 to 12 turns of the spiral With dark ground appears as silvery delicate corkscrew in motion Imperfect illumination may show them as lots Curves rigid while in *Borrelia* they tend to straighten out Members of



Pl. 72 Diagram of the spirochetes. The first is the *Spirochaeta*, the second is the *Treponema*, the third is the *Leptospira*, and the fourth is the *Craspedosporon*. (After Noguchi, Journ. Biol. Med. 1917)

both these genera (German theory) composed of ecto and endoplasm the former being continuous beyond the latter forming the attenuated ends

6 *Leptospira* Noguchi 1917 type *L. interrogans*—Inala and Ido 1914 14  $\mu$  in length 0.3  $\mu$  in width with pointed ends and a spiral amplitude of 0.45  $\mu$  none more gently undulating curves Terminal filament axial filament undulating membrane absent Resist 10 per cent saponin but are dissolved by bile salts Three pathogenic species *L. icterohaemorrhagiae* *L. hebdomadis* and *L. morum* Progress by rotary motion with one end hooked

The first 3 genera do not occur in man and are of no medical significance The members of the genus *Leptospira* are often referred to as the blood spirochaetes in contrast with the *Treponema* or tissue spirochaetes Intermediate between these

A cord of *S. m.* (1930) the sp. is 1.5  $\mu$  in diameter and persists in the blood for several weeks in 10 per cent saline solution with *S. pallida* and *S. icterohaemorrhagiae* are readily attacked

an axial filament. Obermeier in 1873 noted the occurrence of filamentous spiral forms in the blood from patients with relapsing fever. In 1903 Schaudinn and Hoffman discovered the organism causing syphilis and named it *Treponema pallidum* regarding it as a protozoan. Hitherto the spirochaetes had been classified as bacteria. Many other protozoologists have followed the classification of Schaudinn and described the spirochaetes among the protozoa. Today while there is not entire agreement upon the matter it is the consensus of opinion that they are more nearly related to the bacteria. Nevertheless they have characteristics common to both and individual properties which distinguish them as a class.

All the pathogenic spirochaetes are more flexible than bacteria though their flexibility varies with the different species. Thus the spirochaete of rat bite fever (*Leptospira morsus muris* or *Spirillum minus* (Carter 1887) may show great rigidity in its spirals.

Like bacteria spirochaetes lack any distinct nucleus and divide exclusively by transverse division usually into two equal forms. It was formerly believed that they multiplied by longitudinal division but the figures interpreted as indicating longitudinal fission are now considered to have been probably  $\lambda$  or  $\gamma$  forms produced by bending at the point of division of partially intertwined spirals. They do not form spores. Granular forms have been observed and described as representing a stage in the life cycle of the organism. Filtrates containing such granules have been shown to be infective but since many of the flexible spiral forms may also work their way through bacterial filters the assumption that the granules are infective and are a phase in the life cycle is not thereby proved. Indeed today the infectivity of the granular forms is still questionable.

The spirochaetes are motile having sinuous and rotating movements which are independent of the action of the flagella. Many observers believe they have no true flagella. Delicate terminal filaments however have been observed in some and in the case of the organism of rat bite fever terminal flagella are present.

Spirochaetes differ from bacteria in being susceptible in the animal body to the destructive action of arsenic, antimony, bismuth and mercury compound. One of the most striking evidences of this fact is seen in the treatment of yaws with salvarsan.

The spirochaetes usually stain with more difficulty than bacteria and are most effectively stained by methods such as Wright's or Giemsa's. They are gram negative. For staining in the tissues Levadanti's method is usually most satisfactory. For the staining of film preparations the Fontana-Tripodéau silver method gives good results.

Some of the species are aerobic and others are anaerobic. Cultivation is difficult and often unsatisfactory. However cultures have been obtained of most strains in media to which serum or blood and sometimes fresh tissue has been added. The organisms often lose their pathogenicity rapidly in cultures. In the living state they are often seen best by the dark field illumination.

Noguchi proposed a classification of the spirochaetes based largely upon morphological differences (Fig. 72). The following classification adopted by Bergey is based upon that of Noguchi but the name *Borrelia* is used for the genus which Noguchi originally termed *Spirochaeta*. However the names *Spirillum* and *Spirochaeta* were earlier applied to this genus.

### SPIROCHAETACEA

1 *Spirochaeta* Ehrenberg 1833. Large free living fresh water and marine forms. Type *S. plicatilis* ( $500 \times 0.75\mu$ ) cylindrical with regular spirals  $1.5\mu$  apart. Has an elastic flexible axial filament but no crista or flagella. Not dissolved by bile salts or saponin in 10% solution.

2 *Saprosipira* Gross 1911. Large free living marine and fresh water forms. Type *S. grandis* ( $100 \times 0.8\mu$ ). Is divided internally into chambers by many transverse septa. Organism disposed in numerous relatively rigid undulating curves. There are no flagella nor is there an undulating membrane (crista).

3 *Cristispira* Gross 1910. Large spirochaetes parasitic in alimentary tract of oysters and other shell fish. Type *C. b. b. an.* Certes 1882 ( $45$  to  $90 \times 1.8\mu$ ) with

## THE RELAPSING FEVERS

**Definition**—Relapsing fever is a febrile arthropod borne spirochaetal infection which is widely distributed in many parts of the world sometimes producing wide spread epidemics. The disease is characterized by a short febrile period (4-10 days) which begins and ends abruptly and is usually followed after a week or two by a similar but milder paroxysm. In the African tick fever there may be as many as 10 such relapses whereas in the European type of the disease there are rarely more than 2 or 3. With each relapse there is a fresh invasion of spirochaetes into the blood stream where they persist until shortly before the crisis. During the remissions the organisms practically disappear from the peripheral blood but they may be found in large numbers in the hyperplastic spleen in which they are apparently harbored. The cerebro-spinal fluid may sometimes become infected and the spirochaetes have been demonstrated there by animal inoculation up to 45 days after their disappearance from the blood.

Relapsing fever has occurred in all the continents of the world with the possible exception of Australia. It is common in Eastern Europe and many great epidemics have occurred especially in the Balkan countries Austria and Russia. In Africa it probably ranks in prevalence next to malaria and sleeping sickness and in India it is also particularly severe.

There is no significant clinical difference in the disease as it occurs in the various countries. In some regions however it is spread by infected lice and in others by infected ticks. Both louse borne and tick borne cases have been reported from the same regions in Africa and in South America. It has been shown that these spirochaetes can live in the bodies of bed bugs and it is quite possible that these hemiptera sometimes may also act as vectors.

The spirochaetes causing relapsing fever have been differentiated (see p. 323) into several more or less distinct types of species of which the more clear cut are *S. recurrentis* causing the (louse borne) European disease and *S. duttoni* causing African tick fever. Morphologically similar organisms are responsible for various arthropod borne blood infections in fowls cattle and other animals.

## HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History**—Although Hippocrates described the clinical features of relapsing fever quite accurately this knowledge seems to have been lost until about the 18th century. The disease described by Hippocrates as existing at Thasos may have been a form of malaria. References to relapsing fever go back to 1741 when Rutty reported a disease of this type associated with typhus fever in Dublin. The name relapsing fever seems first to have been given and the disease clearly differentiated from typhus fever by Henderson who described the clinical differences between the 2 diseases in an epidemic at Edinburgh in 1843. Craigie also described this epidemic. Griesinger's bilious typhoid of Egypt (1851) was probably

genera is the *Leptospira* group the members of which have characteristics in common with both. The *Borrelia vincenti* however has not been demonstrated in the blood and its inclusion in this genus is questionable. The nomenclature of the organism causing rat bite fever is also unsatisfactory. Although included here in the genus *Leptospira* (*Leptospira morsus muris*) it differs morphologically from other members of the group in the rigidity of its spirals. Unlike other spirochaetes it possesses terminal flagella and its motility resembles that of the vibrios. On this account many bacteriologists consider that it should be placed in the genus *Spirillum*. Rat bite fever however resembles other spirochaetal infections clinically and responds in the same way to therapy with the heavy metals.

This classification adopted by Bergey is in some respects far from satisfactory and especially with reference to the distinguishing of *Spirochaeta* and *Borrelia*. The first generic name used with reference to this group of microorganisms (the spirochaetes) was *Spirochaeta* Ehrenberg (1839). Although Ehrenberg first applied it to large free living forms Lebert (1874) who described the spirochaete causing relapsing fever in man gave it the specific name of *recurrentis*. Such terms as *Spironema*, *Treponema*, *Leptospira* and *Borrelia* have been applied either to replace or subdivide the genus *Spirochaeta* or as additions to it. However *Spironema* had been preoccupied both in zoology and botany. Blacklock (1938) points out that many authorities today are of the opinion that these later names cannot stand as the supposed characteristics upon the existence of which they were established may not exist and at best are unreliable. In a number of recent text books following the suggestion of Zuelzer and Mesnil there has been a return to the old nomenclature and it hence seems most advisable and particularly on account of common usage to retain in medical literature the generic name *Spirochaeta* in order to avoid further confusion.

There are at least 4 species of spirochaetes which are distinguishable and definitely known to be pathogenic for man. These are *Spirochaeta recurrentis* Lebert (1874) which produces relapsing fever transmitted by the louse and morphologically identical with *S. duttoni* the form transmitted by ticks, *Spirochaeta pallida* Schaudinn (1905) (*Treponema pallidum*), which produces syphilis and morphologically identical with *S. pertenue* which causes yaws, *Leptospira icterohaemorrhagiae* which causes haemorrhagic jaundice and *Leptospira* or *Spirillum morsus muris* which causes rat bite fever.

In different parts of the world the following names or synonyms have been applied to the organisms causing relapsing fevers in man

	<i>recurrentis</i>	Europe
	<i>obermeieri</i>	Europe
	<i>duttoni</i>	Central Africa
	<i>crociduri</i>	Africa
	<i>novyi</i>	America
	<i>carteri</i>	India
Spirochaeta	<i>kochi</i>	East Africa
Spironema	<i>rossi</i>	East Africa
Treponema	<i>brerum</i>	North Africa
Borrelia	<i>persicum</i>	Persia & N W Africa
	<i>aegypticum</i>	Egypt
	<i>venezuelense</i>	Venezuela Colombia
	<i>neotropicalis</i>	Panama
	<i>hispanicum</i>	Spain
	<i>morocana</i>	Morocco
	<i>sogdianum</i>	North Africa
	<i>tumacatae</i>	Texas
	<i>latyshewi</i>	Middle Asia

ing fever to Philadelphia. The disease was also observed in groups of immigrants in New York in 1848-50. The only wide spread American epidemic occurred in 1869-71. It was then very prevalent in New York and Austin Flint studied over 100 cases in the wards of Bellevue Hospital. In Philadelphia there were over 1000 cases and scattered cases were observed in Washington D. C. Maryland New Jersey and Connecticut. In 1870 William Pepper studied and later wrote an excellent clinical description of the disease based on 200 cases in the Philadelphia hospital. Since that outbreak apparently only occasional isolated cases of louse borne disease have occurred in the Eastern United States. Palmer and Crawford in 1923 could find a record of less than 20 cases since 1875.

Relapsing fever transmitted by ticks is more prevalent in Africa than in any other country where in prevalence it ranks next to malaria and sleeping sickness. Throughout tropical Africa the distribution of the tick *Ornithodoros moubata* coincides with that of the disease. According to Bequaert the majority of specimens of this tick in Africa are infected with the spirochaete. In localities where the tick occurs the adult natives have often become immune to the disease but new comers when bitten hardly ever fail to contract the fever.

Foci of tick fever often transmitted by other species of ticks are also present in Spain North Africa Arabia Persia India and other parts of Central Asia as well as in Syria more recently (1933) in Cyprus.

In America in recent years epidemic foci of infection of tick borne relapsing fever in which different species of *Ornithodoros* are the vector have been found in

Southern California Colorado Arizona Texas Kansas Idaho New Mexico Nevada Oklahoma Oregon Washington Utah Mexico Panama Cuba Colombia and Venezuela Peru Uruguay and the Argentine. In 1934 Palmer and Crawford reported 6 cases of tick borne relapsing fever in the Arrow Lake region of British Columbia. In California Miller in 1875 reported upon an epidemic of relapsing fever among Chinese laborers at Oroville which prevailed during the months of August September and October and it was estimated that there were several hundred cases. The disease was not noted again in California until 1906 when two deaths were recorded. During the next 15 years occasional cases were reported and in 1921 Bright demonstrated spirochaetes in the blood in patients from near Lake Tahoe.

From 1921 to 1938 138 cases were demonstrated especially by Wynns and Beck and Wheeler. In Texas from 1930 to 1935 158 cases were observed and studied especially by Weller and Kemp and their associates. Apparently the first cases of relapsing fever in the western United States occurring in native Americans in whom spirochaetes were demonstrated in the blood were reported in Colorado in 1913 by Meader.

#### ETIOLOGY

All the forms of relapsing fever which occur in different parts of the world are caused by a species of spirochaete present in the blood of the

Davis et al have now reported spirochaetes in *Opiliones* in addition in Montana and Wyoming.

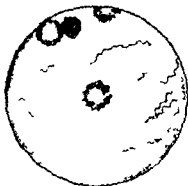


FIG. 73.—Spirochaetes (relapsing fever) from blood of man (Kolle and Warrington).



relapsing fever and it is noteworthy that in fatal cases of this disease occurring in the Gold Coast in 1857 he noted and described miliary lesions of the spleen which we now know are commonly present in fatal cases of relapsing fever

The causative spirochaetes were first seen by Obermeier in the blood of a patient in 1868 but he did not publish his discovery until 1873

During that year he found spirochaetes in a large number of patients during an epidemic in Germany Munch (1874) infected himself and Motschutkoffsky (186) produced relapsing fever in normal human beings by the injection of blood containing the spirochaetes In 1877-78 Carter proved that the disease in India could be similarly transmitted to man and monkeys by the injection of an infected patient's blood In 1904 Ross and Milne working in Uganda demonstrated that the disease known there as tick fever (and supposedly transmitted by the bite of this arthropod) was likewise due to a spirochaete found in small numbers in the circulating blood Although the disease termed tick fever had been recognized for a long period of time and was mentioned by Livingston in 1857 its etiology had previously remained obscure In 1905 Dutton and Todd working in the Congo were able to confirm the observations of Ross and Milne and show that the parasite could pass into and was present in the ova and larva of the tick *Ornithodoros moubata* (Murray) and so confer infecting power upon the mature form of the succeeding generation

Flügge in 1891 suggested the louse as a carrier of relapsing fever and Mackie (1907) after an epidemiological survey of an epidemic in India concluded that the louse *Pediculus humanus* was the vector Experimental confirmation of this idea was given by Sergent and his associates (1910-11) and later Nicolle Blaisot and Conseil (1912) who reported the infection of healthy monkeys by means of the inoculation of body fluids of crushed lice which had been taken from patients with relapsing fever In recent years a number of different species of ticks of the family *Argasidae* have been shown to transmit the disease in different parts of the world

**Geographical Distribution**—Relapsing fever transmitted by *Pediculus humanus* is endemic and frequently becomes epidemic in Europe parts of Asia and Africa sporadic cases are occasionally observed in the United States In Europe it occurs from time to time in the British Isles especially in Ireland and in Norway Denmark Germany Poland Russia the Balkan States and Turkey It has often been the scourge of armies in the field and during the World War it was prevalent in Serbia where it was sometimes found to co exist with typhus fever Over 12 000 cases of relapsing fever were diagnosed in Serbia in 1915

In Asia it is especially met with in India where large epidemics occurred in 1922 in the Central Provinces and the Northwestern Frontier It also prevails in parts of China Manchuria and Persia Although it has been stated in some text books to occur in the Dutch East Indies De Langen and Lichtenstein (1936) state the disease has not yet been observed in the East Indies and Scott (1939) says in Australia there is no known relapsing fever In Africa it is found especially in northern Africa (Egypt Algeria the Sudan [at intervals] and Abyssinia) and in Western Africa the Gold Coast Nigeria Senegal and the French Sudan In recent years it has been especially prevalent in Dakar Cooper (1941) has reported an outbreak in Tobruk in which there were 63 cases

In the United States in earlier years the louse transmitted disease was imported on a number of occasions by patients arriving especially from the British Isles

During the years 1842-52 the disease was prevalent in Ireland Scotland and England In June 1844 an immigrant ship from Liverpool brought 18 cases of relaps

**Immunity**—With recovery from the disease a transient immunity develops and the serum acquires the ability to protect animals from subsequent inoculation. Bactericidal and agglutinating antibodies can be demonstrated. When spirochaetes in the blood are treated with an immune serum they generally undergo lysis and soon only granules remain. Relapses are frequent but are ordinarily less severe than the original attack and may be explained by an inadequate formation of antibodies. The more resistant spirochaetes survive and multiply and again invade the blood stream. With each relapse the immunity is gradually increased until recovery is complete. The spirochaetes have been found in the blood of individuals with only slight symptoms and such cases may be of great importance in disseminating the disease.

Francis (1938) found that 6 rhesus monkeys that had recovered from the disease did not show any immunity against a reinfection 9 months to a year later. On the other hand Lawrence found that in cases experimentally infected by ticks who had recovered he was unable to reinfect them for as long as two years afterwards. Sagel found patients immune who had been infected with *S. duttoni* for as long as five and one half years after recovery.

**Differentiation of Species**—A great many attempts have been made to distinguish different species of the spirochaetes of relapsing fever by the use of immune sera. The writer found (1909) that agglutinative and bacteriolytic reactions were not satisfactory for the differentiation of species since agglutinines and bacteriolytins or lysins are present in varying amounts in the animal's blood during the different stages of the infection which may alter the reactions of the invading spirochaetes. The most satisfactory method of differentiation is through the thorough immunization of animals each with a different strain. Thus white rats thoroughly immunized by repeated inoculations of *S. recurrentis* of European relapsing fever were subsequently found to be still susceptible to infection with *S. duttoni* of African tick fever.

However it was not possible by such immunization reactions to differentiate between *S. recurrentis* and *S. carteri* and *S. morrisi* as was shown by Mackie and the writer. It is not clear that the strains termed American and Indian are serologically different from the European. If they are not identical they must be closely related. Manson Rahr (1935) in his table illustrating the different strains of spirochaetes only differentiates according to serum reactions *S. recurrentis* and *S. duttoni*. Nicolle and Anderson (1932) consider that the relapsing fever spirochaetes seem to fall into 2 groups: a very homogenous *S. duttoni* group all strains of which are serologically identical and a second group in which there is a tendency to break up into different races.

Kemp, Moursund and Wright (1934) found the Texas strain was immunologically identical with *S. morrisi*. However they found that in some experiments with *O. turicata* which had been infected with strains termed *S. morrisi duttoni* and *recurrentis* that this tick did not transmit the infection to rats. Brumpt on the basis of such results, namely that only the Texas strain developed in *G. morsitans* named it *S. turicatae*. However these investigations should be continued until such different strains might be merely one of tolerance or of adjustment to a new environment in another species of tick. Craig and Lawton (1931) suggest that the following designation *R. turicatae* is preferable to *S. turicatae*.

Russell (1933) in Africa and Cunningham (1935 and 1936) Theodor and Fraser (1936) in India have found that the spirochaetes present in the primary attack give different serological reactions from those found in the second and third attacks which obviously greatly complicates any differentiation of species by serum reactions. Not only does the serum become altered by the presence of immune bodies early in the disease but the organisms themselves become serologically changed by it. It is not unusual to see agglutinated organisms in the blood. Nevertheless, as has been noted

type *S. recurrentis*. However, the many different strains or so called species (listed on p. 324) are morphologically indistinguishable from one another. The parasite is usually demonstrable in the blood only during the febrile stages of the disease and it often disappears 48 hours before the crisis. A short time before the crisis however the organisms may sometimes be seen agglutinated in small clumps or star shaped forms. In some instances the spirochaetes are present in large numbers and easy to detect. In other instances many microscopical fields must be examined before the parasite is found.

**Morphology**—The *Spirochaeta recurrentis* varies in size. The different forms measure from 10 to  $30\mu \times 0.4\mu$ . They are flexible and have from 4 to 10 open irregular coils. The ends are tapering. They have an active corkscrew motility in fresh blood preparations. They are stained by the usual bacterial stains and especially by the Romanowsky blood stains as well as by silver impregnation methods. Individual organisms may have a beaded appearance although a majority of them stain uniformly.

The organism is found in man and animals in the blood stream and cerebro spinal fluid. At autopsy it is usually especially prevalent in films made from the spleen and sometimes from the liver. In the insect vector the louse *Pediculus humanus* it is found first in the gut and later in the haemocoel. In the tick it is found first in the gut later in the haemocoel and later in the tissues and organs including the ovaries and ova.

Leishman first described a breaking up of the spirochaetes in the alimentary tract of the tick into small granules which penetrated the malpighian tubules and the ovary. He regarded these granules as the infecting agents and suggested that they represent a phase in the life cycle of the spirochaete. This hypothesis has received approval from a number of investigators. However according to Wenyon and Southwell (1938) it has not been definitely substantiated and the infectivity of the granules must still be regarded as *sub judice*.

**Cultivation**.—Noguchi succeeded in obtaining cultures of this organism by using a medium containing ascitic fluid, blood and a bit of fresh sterile tissue and incubating them anaerobically. Cultures have since been obtained in media enriched with serum or blood without the addition of fresh tissue. However cultivation is sometimes unsuccessful and it is not a practical diagnostic procedure. Several workers have reported successful results with egg media. Manteufel and Dressler (1933) report excellent results with pieces of allantoic membrane in Tyrode solution in which strains of *S. hispanica* remained virulent for at least 38 passages.

Oag (1939) and others have reported that *S. duttoni* may be cultivated in developing fowl embryo. Oag has cultivated a strain continuously for 10 passages over a period of one month and obtained abundant growth. The spirochaetes invaded the blood stream of the embryo.

Chen (1941) has also cultivated the louse borne strain from 7 human cases by inoculating the spirochaetes into the chorioallantoic membrane of hens eggs. A maximum growth is obtained by the fifth day.

**Animal Inoculation**—Monkeys, mice and rats can be infected by subcutaneous inoculation. The disease produced in monkeys resembles the human infection and the spirochaetes are demonstrable in large numbers in their blood during the febrile period. The blood of infected mice and white rats also contains the organisms. Guinea pigs are usually resistant to infection with many strains.

The strains *S. cecidurae* first found in rodents and inoculated into man and *S. normandi* also of rodents are now regarded as identical with *S. d. lionsi* and transmitted by *Ornithodoros*.

#### TRANSMISSION

**Louse borne Relapsing Fever**—The European Indian Chinese West African and some of the North African infections are especially transmitted from one individual to another by the louse *Pediculus humanus*. After biting an infected person the spirochaetes which are taken into the alimentary tract of the louse disappear within 24 hours. The insect is apparently harmless for the succeeding 4 or 5 days. At the end of this time spirochaetes reappear in the haemocoel fluid of the louse which then remains infectious for 2 or 3 weeks. It has been stated that organisms may be present sometimes in the excreta of the louse through which the bite wound may be contaminated. However some observers believe that contaminative infection by the agency of the faeces does not occur (Blacklock 1938). Nicolle and others were able to produce the disease in animals by rubbing the abraded skin with an emulsion of crushed infected lice after having shown that infection was not produced by their bites or by the injection of their faeces. It is generally accepted however that the disease in man generally is caused by contamination of a bite wound or a scratch by the material from a crushed louse.

Chung and Wei (1938) transmitted the Chinese strain of *S. recurrentis* to 6 normal subjects and 4 patients with general paralysis of the insane. Five subjects were exposed to the bites of large numbers of human lice known to be infected but no signs of infection developed. Positive results however were obtained when 4 lice infected 12 days previously were ground up in saline and this emulsion was placed on the skin of the forearm of a man who had just been bitten on the same place by normal lice. This patient became infected with relapsing fever after 11 days incubation period. In one experiment the faeces of 100 lice collected daily 7 to 12 days after an infected feed were instilled onto the excoriated skin of a volunteer but no fever was observed during 6 weeks. The results of these experiments support the view that the bites and faeces are innocuous and the disease is contracted through crushing infected lice on the skin.

Brumpt (1936) believes that while tick borne relapsing fever has rodent reservoir hosts the louse borne disease has no host but man. Nevertheless the Mediterranean form of relapsing fever due to *S. hispanicum* it is stated by delBuen (1936) and Nicolle and Anderson may be experimentally transmitted both by the louse and by the tick.

**Transmission by Ticks**—Other types of relapsing fever are transmitted by ticks especially of the genus *Ornithodoros* of the family 1RG 1SID 1F

Tick borne relapsing fever occurs especially in southeastern and Central Africa where *O. moubata* is the important transmitter or the very closely related *O. savignyi* and in Tunis where *O. erraticus* is incriminated. In Spain and Morocco where *O. m. a.* is involved and in Central Asia and Palestine where *O. papillipes* or *O. tholozani* (Iran) is the transmitter. In northern South America and Panama Central America

above a considerable number of strains of the parasite have been described under different names and as new species. In some of these different serum reactions have been reported and a number of them are known to be transmitted by different species of blood-sucking arthropods. Schuhardt has found that in the tick borne *S. turicatae* strain the spirochaetes developing at the onset or at relapses consist of multiple antigenic varieties which undergo further alteration in rats as antibodies are developed against them. Brumpt suggested that it might be better that the spirochaetes should be classified on the basis of their arthropod hosts. Davis (1942) believes that a specificity exists among *O. turicata*, *O. parkeri* and *O. hermsi* and their respective spirochaetes.

The most important of the relapsing fevers in epidemic form is the louse borne one of Europe due to *S. recurrentis*. *S. berbera* of the North African disease and *S. carteri* of the Indian relapsing fever are transmitted also by the louse *Pediculus humanus* as is the strain producing relapsing fever in China. Apparently no experi-

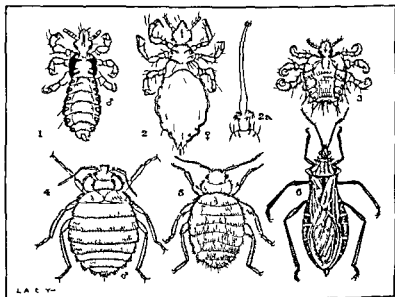


FIG 74—Anoplura and Hemiptera. 1 *Pediculus humanus*. 2 *Pediculus testaceus*. 2a Protruded rostrum of *Pediculus*. 3 *Phthirus pubis*. 4 *Cimex lectularius*. 5 *Crotalaria*. 6 *Triatomina*.

ments were made regarding the transmitting arthropod of the original strain *S. novyi* found in one case in the United States in a ship's steward who had returned recently from tropical America.

*S. duttoni* the organism encountered in the severe Central African tick fever is transmitted by *Ornithodoros moubata*. *S. persica* found in the Persian disease and northwest India and *S. hispanica* of South Spain and Morocco are transmitted by ticks of the genus *Ornithodoros* but according to Delanoe the Moroccan spirochete differs slightly and he has proposed the name *S. marocana* for it. It is transmitted by *O. marocanus* or *O. erraticus*. de Buen and Nicolle, however, have found that the Spanish form may also be transmitted experimentally in animals by the louse.

The South American forms due to *S. venezuelense* and *S. neotropicalis* of Panama are also transmitted by ticks *O. venezuelensis* and *O. talaje* and the Texas strain termed *S. turicatae* by *O. turicata*. However the Panama strain is not transmissible by *O. turicata* and the Texas strain not transmitted by the ticks which carry the tropical Panama strain. In California the strain encountered is said to be immunologically close to the Texas form but its transmitter is *O. hermsi* and it seems not to be transmissible by *O. turicata* (Chandler 1940). Mazzotti (1943) says *S. venez.* be transmitted by *O. parkeri* or *O. hermsi*.

they did not think the spirochaetes survived there for long periods and they did not find them in the malpighian tubes and salivary glands. The bites of the infected bed bugs were not infectious for squirrels. Francis (1938) has found that white mice may become infected following the ingestion of infected bed bugs and obtained successful results in 4 of 8 experiments when the bugs were fed to white mice; the incubation periods in the mice being from 4 to 6 days. It was found that the white mice readily attacked and ate the bed bugs.

Francis found that it was not possible to transmit infection from monkey to monkey by means of the monkey louse *Tedius longiceps*.

**Other Means of Infection.**—It is recognized that the spirochaetes of relapsing fever are capable of penetrating mucous membranes as the conjunctiva and there have been a number of reports of infection occurring through the entry of infected blood by accident into the conjunctival sac.

Chung and Wei (1938) have found that by placing the contents of body lice fed on cases of relapsing fever into the conjunctivae of 3 human individuals, 2 of them became infected with relapsing fever after an incubation period of 8 days. L. Abbate and Manino (1938) have reported a case in which they believe transmission occurred by infected blood having entered the nostril of one of them. In some instances it has been reported that the parasite apparently might even pass through skin that was apparently normal in appearance. However in other instances when a large number of spirochaetes were put on the normal skin infection did not occur. Thus Chung and Wei (1938) on occasions in which large numbers of *S. recurrentis* were dropped upon the skin of a patient with general paralysis of the insane found that infection with relapsing fever did not follow. They also made various attempts to infect patients by placing the contents of infected lice in the mouth which gave uniformly negative results. However J. Jeux and Sautet (1938) have reported that infection has been produced in rats by feeding them with the brains of other rats infected with *S. duttoni*. They found the infection produced by feeding had a longer incubation period (10-14 days) than when infection was produced by *Ornithodoros*. Francis as noted above also found that white mice became infected from eating infected bed bugs.

Both in pregnant women and pregnant animals the spirochaetes have been shown to be capable of passing through the placenta and infecting the foetus.

**Relationship of Tick borne to Louse borne Relapsing Fever**—It has been stated that tick borne relapsing fever is not infective for the louse (Faust 1937). Feng and Chung (1937) support this view and found that *S. recurrentis* (a Chinese strain transmitted normally by lice) cannot be maintained in very young or adult *O. moubata* and they suggest that *S. recurrentis* is biologically different from the strain transmitted by the tick. However Mantefel (1908) found that he was able to transmit the European strain of relapsing fever (a Russian strain) to rats through the bite of experimentally infected *O. moubata* and Neumann (1906) reported success in transmitting strains described as American and Russian of *S. recurrentis* to mice and rats through the bites of the same tick. Nicolle in studying the Algerian form *S. berbera* and the Sudanese *S. argyrophorum* which are louse borne was able to transmit the infection by the tick *O. morsus* which infested the burrows of a number of rodents and he also transmitted the infection by this tick to mice and rats. Also the Spanish form of relapsing fever is known to be transmitted by *O. morsus* and *O. erraticus* as has been shown by deBuen in 1936.

where *O. venezuelensis* and *O. talaje* are concerned in Mexico Texas Kansas and Oklahoma where the species *O. turicata* transmits the infection and in California where *O. hermsi* is concerned. In these ticks the spirochaete is congenitally transmitted from the adult female tick to the offspring and sometimes to the third generation. One case of human infection has been reported attributable to the tick *O. parkeri* Davis (1941). T. Mazzotti (1942) reports personally that *O. venezuelensis* so far has not been found in Mexico and not in Central America except Panama.

The tick after feeding upon an infected human individual ingests the spirochaetes which quickly diminish in number and disappear from the gut passing through the gut wall. They enter the haemocoel and become distributed in different parts of the body particularly in the cells of the gut the malpighian tubules coxal glands and salivary glands. They have often been observed in the legs of the insects. In some instances, the ticks may be infective within an hour after the ingestion of blood containing the parasites. The parasites later multiply and after some weeks may be found in considerable numbers in the haemocoel fluid.

The most common method of infection of man by the tick is disputed. Hindle (1931) and Brumpt (1936) regard both the coxal fluid and the faeces as infective while Manson Bahr (1936) appears to regard the faeces as the chief source of infection. Blacklock and Southwell (1938) state the infection is usually held to be contaminative but the inoculative method by infected salivary fluid has been proved for *S. sogdianum*. Bonné (1938) who has performed numerous experiments in transmission with *O. moubata* believes that the salivary glands do not become infected. He obtained negative results from the bites and also with the faeces of the tick and he considers that the coxal fluid alone is responsible for the production of infection.

Wheeler (1938) however made experiments on monkeys and human beings with *O. hermsi* and found that in the positive cases the infection occurred from the bites of the ticks via the mouth parts as no coxal fluid or faecal material was exuded by the ticks either while they were biting or immediately after detaching them. Francis (1939) examined on many occasions the coxal fluid which was secreted at the time of feeding of *O. turicata* for the presence of spirochaetes with negative results nor could spirochaetes be demonstrated in white mice into which coxal fluid was injected. Feng and Chung (1938) found that natural transmission of *S. duttoni* to mice by *O. moubata* might be produced by the bite of ticks alone before coxal fluid was passed and by both the bite and coxal fluid together but that the faeces do not contain spirochaetes and are not infective. Francis (1938) has found that relapsing fever spirochaetes will survive within ticks of the species *O. turicata* for as long as 6½ years. Previous investigations have shown that the species *O. moubata* may harbor living virulent spirochaetes for over a year. When ticks are kept at low temperatures the spirochaetes often fail to increase sufficiently to render them infective but if kept at 30–37° for a few days they then may become infective and retain such infection even at temperatures of 5–8° C for 2 months.

**Bed Bugs**—Transmission by the bedbug *Cimex lectularius* has been suggested by a number of investigators. Tictin (1897) demonstrated that the European infection may be transmitted to monkeys by inoculating the contents of crushed bed bugs which had fed on a patient with relapsing fever. Nuttall also transmitted *S. recurrentis* from mouse to mouse by crushed bed bugs. However Dunn (1923) failed in animals to produce infection with bed bugs except by inoculation of the contents of the crushed bugs. Rosenholz and Francis (1932) found that *S. recurrentis* would survive in bed bugs for over 5 months. They obtained transmission to white mice by injection of the bugs 190 days after their infective feed but failed to obtain transmission by allowing the bugs to feed on white mice. Chung and Feng (1938) found that the gastric juice of the bed bug appeared to be detrimental to *S. recurrentis* most of the spirochaetes ingested being killed within 24 hours though occasionally a few were found to survive for 2 days in the stomach. They however found the parasite in the legs and coelomic fluid of bugs as early as 1½ hours after having fed on a patient with relapsing fever. Still

times with a pulvillus. The nymph has stigmal plates but has no genital opening while the larva has neither genital apertures nor stigmal orifice.

**Life History.**—This varies with different ticks. That of *De macentior* may be taken as representative of the group. After the adults have succeeded in reaching a suitable host they engorge, mating occurs and they drop to the ground. The males die at once. The females deposit their eggs in some protected place away from the host and then they also die. From 2000 to 8000 eggs are deposited in the course of a month. (The number of eggs varies from 1 or 2 hundred in the Argasidae to 20 000 in certain of the Ixodidae.) After a period of development of a month or more a small 6 legged larva (seed tick) emerges. This crawls up a blade of grass and waits until it can attach itself to some passing animal. It then engorges and within a few days drops to the ground. Here it undergoes further development for several weeks, finally moulting and becoming an 8 legged nymph. This in turn climbs up a grass blade or a twig and awaits another passing animal (the second host). If fortunate enough to reach one it engorges and again drops to the ground and after several weeks development moults and becomes a mature adult. This must gain access to a third host in a similar manner to complete the cycle. A female tick may ingest 100 times its weight of blood.

The length of the life cycle varies greatly with the species and with weather conditions. In the case of *D. andersoni* it is over 2 years, the tick passing the first winter as an unfed nymph and the second winter as an unfed adult. In other species the cycle is completed in a season. Winter may be passed in the egg stage. The extraordinary capacity of the ticks to survive starvation compensates in part for the uncertainty and frequent delay in reaching a host. Larvae have survived to 8 months and adults 3 to 5 years without food.

Many variations from this type of cycle have been observed. Among the Argasidae the adults often engorge and mate several times on different hosts. In some Ixodidae the larvae and nymphs and even the adults also may complete their development on a single host, feeding only for oviposition (two host or one host ticks). In some species the males do not bite and their mouth parts are rudimentary.

### CLASSIFICATION OF IKODOIDEA

**Family Argasidae.**—Head concealed by body when viewed dorsally. No scutum. Stigmal plates between third and fourth legs. Adults have no suckers (pulvillus) beneath claws. Slight sexual dimorphism. Anus near middle of venter. Skin rough.

These ticks are chiefly parasites of birds, bats, etc. and occasionally of man living and breeding in the nests or lair of the hosts to which they have relatively ready access.

**Genus Argas.**—Body narrow in front. Margins thin and sharp. No eyes. Rostrum some distance behind anterior margin of body. It is the common fowl tick (*Vianna bug*) transmits sp. fulvus of fowl. It has been suspected of transmitting a form of relapsing fever in India.

**Genus Ornithodoros.**—Body oval margins rounded. Skin has many irregular tubercles. Rostrum close with anterior margin of body so that ends of palpi project slightly.

### THE MOST IMPORTANT TICKS CONCERNED IN TRANSMISSION

*Ornithodoros moubata* (the tampan) is very common in Africa from Uganfa and Somaliland in the east and the Congo and Angola in the West to Namaqualand and through the Transvaal in the South. Bequaert (1930) found no record of its occurrence south of the Orange River. Ordman has found it widely distributed in the northern Transvaal where cases of Relapsing Fever are common. It is also common in Nataland. It has also been found in the North Western coast of Madag-



and monkeys and guinea pigs have been infected by its bite. However if lice were allowed to feed on a monkey so infected such lice when crushed and placed on the scarified skin of normal monkeys, infected them. Nevertheless infected lice were unable by their bites to convey the infection. It hence seems clear that the Spanish form of the disease was transmissible experimentally by both the lice and the tick. However deBuen could find no evidence that the disease was naturally transmitted in Spain by the louse. He believed that this strain of the organism was maladapted to the louse as it had become habitually tick transmitted.

Nicolle and Anderson (1936) have studied the tick borne relapsing fever of Spain transmitted by *Ornithodoros maroccanus* and succeeded in infecting monkeys with this strain in the following way. Guinea pigs and monkeys were infected by spirochete containing ticks. They then had lice feed on these infected monkeys. By taking such lice crushing the bodies and rubbing the material into abraded areas of the skin of other monkeys or by introducing the material into the conjunctival sac they reproduced the infection. Efforts to transmit the disease by natural bites of the lice failed completely. They suggest that relapsing fever was originally endemic and transmitted by ticks but with the development of a new host in the louse it became epidemic and world wide. That the primary vector was the tick of the genus *Ornithodoros* is supported by the fact that many strains of relapsing fever can be transmitted by various species of ticks of the genus.

### IXODIDEA (TICKS)

This superfamily of the order Acarina is of great interest and importance medically and some knowledge of ticks and their identification is of importance from the standpoint of public health.

Ticks differ from insects in having 4 pairs of legs only 2 pairs of mouth parts and no antennae. They differ from other acarines in having a median probe shaped puncturing organ the hypostome which is beset with numerous teeth projecting backward and in possessing stigmatal plates. The head or capitulum or rostrum is the part which projects anteriorly from the body. This carries the piercing parts which are the single hypostome or dart and a pair of piercing chitinous structures the chelicerae which lie above the hypostome. As a sheath for the delicate biting parts there is a segmented pair of palpi or pedipalps. The mouth is a slit between the chelicerae and hypostome.

When the tick reaches a host it first tears a hole in the skin with the chelicerae and then plunges the hypostome into the wound continuing this process until the latter is completely embedded. The recurved teeth anchor this so firmly that if the tick is forcibly removed either the hypostome is torn off and left in the wound or a fragment of skin is torn out with it.

There are 2 depressed pitted areas on the dorsal surface of the capitulum in the adult female known as porose areas. The stigmatal plates are striking mosaic like areas which are located just posterior to each hind leg in the Ixodidae and between the third and fourth legs in the Argasidae. The microscopic structure of the stigmatal plates has been shown by Stil to be of great value in differentiating the various species especially of *Dermacentor*. The stigmatal orifice the opening of the tracheal system is in the center. The Ixodidae have a scutum or shield like chitinous structure on the dorsal surface. It covers almost the entire back of the tick in the male but only a small portion anteriorly in the female. The genital opening is toward the anterior part of the ventral surface. The anus with anterior or posterior anal grooves is near the posterior third of the venter. The legs have 6 segments the coxa being flattened out on the surface of the body and the terminal tarsus ending with a pair of hooks and at

gascar and has been seen south of Lake Chad by Clossel. There appears to be no other record of its existence in the Sudan. It is the only species of the genus known with certainty from the Belgian Congo but it has hardly entered the West African and Congo rain forest where it occurs only in a few of the larger clearings of the Ituri basin.

The females are about 12 mm long; they have a leathery cuticle covered with minute tubercles and flat rounded processes on the legs. There are no eyes. The ticks infest the native huts, particularly the rest houses along the routes of travel, hiding in crevices of the floors and walls during the day and coming out at night to bite the sleeping inmates. Both sexes bite man. It requires more than an hour to engorge. The females lay about 100 eggs. The larva develops to the nymph stage before leaving the egg but the nymphs bite several times before maturity and the adults bite repeatedly.

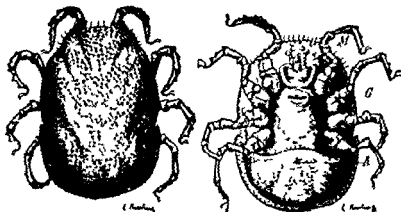


Fig. 6—*Ornithodoros moubati* (Murray from D. B. N.)

This tick is the intermediate host of *Spirochaeta duttoni* (South African tick fever). Both adults and nymphs transmit the infection. The adult may transmit the infection through the egg to the young nymph, even to the third generation according to Möllers. The organisms become distributed throughout the tissues of the tick, including the salivary glands. The infection is conveyed by contamination of the wound with coxal fluid and probably also directly by the bite.

Natives seem to suffer severely from tick fever in childhood but in adult life possess a sufficient degree of immunity so that the disease shows itself in a very mild form in those harboring spirochaetes. Ticks can be infected by such carriers. In some of the rest houses the majority of the ticks may be infected. While the tick does not tend to leave its habit-twin it may be transported in the bundles of native porters.

*O. sanguis*, a closely allied species, differs in the presence of eyes. It has been reported from Egypt, Abyssinia, Somalia, and British East Africa, Rhodesia, Tanganyika, Termit, etc., as well as from South America. It is more diurnal in its habits and has frequently been found in market places and cattle stables. It may be distinguished from *O. moubati* not only by the fact that it has 2 pairs of eyes, pair near the base of the mouth parts and the other between the second and third coxae. It also has larger processes on the legs and a more minute pitted dorsal surface. It has been demonstrated to



to have been introduced by Senegalese troops from Syria \* Over crowding lack of proper personal hygiene especially lack of bathing and general increase and dissemination of vermin common under such conditions—in addition to lack of nourishing food leading to lowered resistance—all contribute to the development of epidemics of relapsing fever Scott (1939) points out that in India epidemics have been frequently started in crowded prisons and spread later to the population outside

The spread of the disease also is especially dependent upon circumstances favoring the *propagation of the intermediate host* either the louse or the tick and its contact with man It is in general the louse borne infection which results in the most serious epidemics and this is especially evident in the history of the occurrence of the disease not only in Europe and Asia but in different parts of Africa and in the United States In the louse borne disease during epidemics there is apparently no mammalian host but man but in tick borne relapsing fever there may also be rodent reservoir hosts

The tick borne disease is most widely spread throughout tropical Africa except in West Africa and most of the Congo rain forest and in Northern Africa But the large and severe epidemics of relapsing fever that have occurred in that continent in recent years have been especially in North and West Africa where the tick *Ornithodoros moubata* is not found or does not prevail Thus a particularly virulent epidemic which commenced in French West Africa in upper Senegal in 1921 and persisted throughout 1924 was apparently louse borne It was thought that infection had been introduced from the Mediterranean area since the first cases occurred at Kouroussa among soldiers from Morocco and Algiers It spread down the Niger to Senegal and the French Sudan and extended southward to the Cold Coast and Nigeria During 2 years the deaths in the French Sudan and the Niger were estimated to have been between 80 000 and 100 000 In 1924 it extended into upper Senegal with 20 000 deaths By September 1926 it had extended to Darfur and in one district alone Atkey (1929) reported 10 000 deaths among a population of 45 000 Altogether it was estimated that 10 per cent of the people died the mortality varying between 5 and 25 per cent According to the reports of Piding and MacDowell (1927) in the outbreak in the western Darfur Province it carried off one fourth of the population the case mortality was said to have been nearly 75 per cent Caffrey (1927) points out that all the evidence was to the effect that the disease was transmitted in Nigeria by the louse *Pediculus humanus* The incidence reached its height in March when the relative temperature and humidity were said to have furnished ideal conditions to favor the bionomics of *Pediculus* Only a few cases were found during the season of heavy rainfall During the epidemic on the Cold Coast in 1924 where *O. moubata* also is not known Ingram proved by experiments on himself and on volunteers that the infection was carried by lice In Nigeria in 1926 there were 814 cases 10 of them fatal and in Uganda the same year more than 1500 cases were reported Epidemics of the louse borne

\* See League of Nations Report 1925 malarious fever occurred in European colonies in West Africa

serve as an intermediate host of *S. duttoni* not only in parts of Africa but also in south western Asia

According to Moise (1938) the tick *Ornithodoros savignyi* transmits the relapsing fever of Abyssinia. However Kirk (1938) was not able to transmit the disease with this tick and believes that the infection there is transmitted by the louse. Spirochaetes were found in the lice fed on infected persons there and the infection was transmitted to monkeys. Also Clinton, Manson, Bahr and Charters (1942) have shown the disease in Abyssinia is transmitted by the louse and is a severe form.

*Ornithodoros talaje* may be distinguished from *O. moubata* as the rostrum of the former may be hidden by the wing like appendages of the comerostoma while in *O. moubata* there are no wing like appendages and the rostrum is not hidden. Also in *O. talaje* the body is attenuated into a blunt point anteriorly while in *O. moubata* the anterior portion is rounded and almost as broad as the posterior.

Candido Carvalho (Jr. *Parasitol.* April 1942) says bats carry this species into the houses in Brazil.

*O. hermsi* has been described as a new species by Wheeler (1935) and is the vector of relapsing fever in California. Our knowledge concerning its distribution is still meagre but it has been collected in the Sierra Nevada and San Bernadino ranges at elevations of from about 5000 to about 8000 feet. It appears to be typically a parasite of rodents but like other species of the same genus feeds freely on a variety of experimental animals as well as human beings. Herms and Wheeler (1936) have taken numerous specimens in all stages of development (except larvae) in the nests of chipmunks in cottages used as summer homes in the mountains.

This species of tick is rather small compared with others of the same genus. The female measures from 5 to 6 mm. in length by 3 to 4 mm. in width while the male is only slightly smaller and resembles the female. It differs from *Ornithodoros talaje* (Panama) a close relative in (a) the absence of large discs on the dorsum (b) the characteristic sculpturing of the integument (c) microscopical differences in the structure of the integument (d) arrangement of the dentition of the hypostome (e) the absence of lateral flap like borders at the margins of the capitulum (f) the shape of the cheliceral teeth (g) the shape of the anal grooves and (h) the tarsi bearing diagnostic protuberances. *O. hermsi* also differs from *Ornithodoros tenebrioides* in (a) the sculpturing of the dorsum (b) the protuberances of the tarsi (c) shape and position of anal grooves and (d) the dentition of the hypostome. It further differs from *Ornithodoros turicata* (Texas) in (a) the absence of clubbed hairs between the mamillae (b) the arrangement and number of teeth on the hypostome (c) the decidedly smaller hypostome (d) the arrangement and number of protuberances on tarsi I and IV in particular and (e) the smaller size of the adults. From observations in the laboratory where the material was kept at a constant temperature and humidity Herms and Wheeler found that the number of eggs deposited per female *O. hermsi* ranges well over 100 deposited at intervals in batches of 12 to 140 from May to October. Davis has found this tick also in Colorado, Oregon, Washington, Nevada and Idaho (personal communication 1944).

## EPIDEMIOLOGY

In Europe relapsing fever has been especially a disease of the poor and more indigenous classes and there have been frequent epidemics during times of famine. Thus it has long been known in Ireland under the name famine fever and some of the earlier Indian epidemics as in 1865-1877 coincided with times of famine. However in other epidemics in India famine has not apparently been an important influence. During recent years wide spread epidemics have occurred among the famine stricken refugees in Central Russia. Nevertheless it should be emphasized that starvation is only one of the predisposing factors and that numerous epidemics have occurred in which there has been no association with general famine among the inhabitants. In war time relapsing fever has sometimes been a scourge of armies in the field. During the War it was prevalent in Serbia and later in West Africa where

In Panama Clark and Dunn have found the squirrel monkey *Leontocbus geoffroyi* naturally infected with *S. ceneruel nusi* which was proved by inoculation to be transmissible to man. About 10 per cent of the specimens of opossum (*Didelph. m. supialis e. tenius*) were also found infected with spirochaetes. *O. talysae* has also been found on this opossum and on *Rattus rattus* and the nymphs may convey the disease from one rat to another. In the southern Belgian Congo also several species of wild rodent have been found to harbor the infection.

Sieff and Gerbils infected in Middle Asia with a species he named *S. talysae* and which he transmitted by direct inoculation to man.

On account of these different factors there is not the same seasonal incidence of the tick borne relapsing fever of Africa as is observed in the louse borne forms nor do they usually assume the epidemic character that has occurred in the louse borne form. It however has been shown by Cunliffe (1921) that an excess of moisture is decidedly unfavorable to the vitality of the tick *O. moubata* and hence it is found particularly in native houses where it is protected against excessive humidity.

**Epidemiology in the United States**—We have recently acquired much epidemiological knowledge regarding the tick borne disease in the United States. In central Texas the disease is transmitted to man by ticks of the species *Ornithodoros turicata* as was first shown by Weller and Graham in 1930. For the 5 year period 1930-1934 Kemp and his associates collected 258 cases in Texas with no deaths though in 1935 and 1936 one death from relapsing fever was reported by the U. S. Bureau of the Census for each of these years. In California 253 cases of the disease have been reported (1921-1941 by Beck). In California the infection is transmitted by the minute tick *Ornithodoros hermsi* (Wheeler) which is no larger than a bedbug. There is considerable variation in the conditions under which these 2 ticks are found in nature.

*O. turicata* transmitting the disease in Texas has been found especially in caves and overhanging ledges produced by water erosion of river banks some of which have a horizontal depth of only a few feet to perhaps 20 feet. They have a ceiling of clay sandstone or limestone which may not be over 4 feet in height. The floor is covered with dry powdery dust or sand which may reach a depth 15 inches. The area close to some of the ledges may admit man and animals freely. Other caves have a horizontal depth of from 50 to 500 feet. The ceilings and walls are of sandstone and may be located several miles from a stream. Ticks located in the dust of the caves have an advantageous position for attachment to the legs of passing animals or of man. The caves are located in proximity to rivers are sometimes subjected to flood water. The ticks are probably transported at times by such water to considerably distant places.

Francis (1939) has carried on laboratory observations extending over 3 years upon ticks collected from such caves in Texas in 1931 and has found that the spirochaetes of relapsing fever have survived within a tick throughout a period of 6½ years. In 14 of the original ticks all females virulent spirochaetes were still present. He has found that these ticks harbored virulent spirochaetes after 5 years of larval and nymphal transmission. Francis (1941) reports that transovarial transmission of spirochaetes has taken place though the generation of offspring taking the tick itself may be a more recent phenomenon than this.

The importance of these observations from the viewpoint of eradication of the disease is apparent since control measures must reckon with ticks hiding in caves and living in rodent burrows harboring virulent spirochaetes in their fatigued bodies for 5 years and in their fecal excreta for 6½ years (and possibly longer) and transmitting the infection through the egg to the next generation of ticks.

(Some experiments with the African species *O. tholozani moubata* have shown that the species which fed from time to time for 71 days living 5 years.)

types have also been recorded by Nogue in French West Africa in 1925 and by McCullough in addition to Caffrey in 1925 and 1926 in the Gold Coast and Nigeria

It has been known that the relapsing fever present in Northern Nigeria and French Equatorial Africa for some years has sometimes arrived there in all probability along the caravan routes from Morocco or Tunis. However, there seems to be some question about the spread of the disease in Dakar. Mathis (1927) who studied the outbreak in Dakar, emphasizes the absence of *Ornithodoros*, but the manner in which the disease was communicated at Dakar was not clear to him. However, a species of spirochaete (*S. crociduræ*) was found in the shrew (*Crociodura stamphii* Jentink, 1887) and in searching the burrows of these animals he succeeded in finding the nymphs of an undetermined species of tick which he thought might be concerned in the transmission. Nevertheless he transmitted the disease from monkey to monkey by means of lice. Other observers had apparently been unsuccessful in finding *Ornithodoros* in Senegal. However Dureaux (1932) succeeded in finding *O. erraticus* in that region. To what extent it comes into contact with man is not clear. The conditions are considerably different regarding the spread of the tick borne forms of relapsing fever in Africa in which the source of the infection is usually confined to houses and similar localities which afford a suitable environment for *Ornithodoros moubata*. This tick infests the rest houses along the route of travel hiding in the crevices of floors and mud walls and thatched roofs during the day and coming out at night to bite the sleeping inmates. The ticks are frequently carried long distances by porters in bedding rolls which have been used and packed in the infected huts. The feeding of ticks sometimes occupies a long time and may not be completed in an hour. Both sexes bite man. The female lays some 50-100 eggs from which the nymphs emerge in about 20 days. The larval stage takes place in the egg. Shortly after emerging the nymphs suck blood. An important fact in epidemiology is that the female transmits the spirochaete to its ova so that the ticks from such ova may transmit the disease.

In recent years it has been recognized that certain rodents may serve in nature as reservoirs of infection of relapsing fever spirochaetes. *Ornithodoros moubata* inhabits at times the burrows of various animals especially the wart hog. Dutton and Todd state that under natural conditions in the Congo this tick *O. moubata* is frequently devoured by rats and mice and it seems not unlikely that infection in rodents might sometimes occur in this manner. Leger in 1917 found in the blood of a shrew in Dakar *Crociodura stamphii* Jentink (1887) a spirochaete to which he gave the name of *S. crociduræ*. This spirochaete which was identical morphologically with the spirochaete of recurrent fever was inoculated by Walker and Marie with success into 2 general paralytics.

Nicolle and Anderson in Tunis also have demonstrated that the spirochaete of rodents described as *S. normandi* is probably identical with *S. duttoni*. This organism is virulent to rats and mice but not pathogenic to guinea pigs but the *hispanica* strain is said to be also virulent for guinea pigs.

Russell in the Gold Coast has found the pouched rat *Cricetomys gambianus* to be very susceptible to infection with *S. duttoni* and *S. crociduræ* has been demonstrated by Mathis and Nicolle and Anderson to be also identical with this strain.

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**Epidemiology in the United States**—We have recently acquired much epidemiological knowledge regarding the tick borne disease in the United States. In central Texas the disease is transmitted to man by ticks of the species *Ornithodoros turicata* as was first shown by Weller and Graham in 1930. For the 5 year period 1930-1934 Kemp and his associates collected 258 cases in Texas with no deaths though in 1935 and 1936 one death from relapsing fever was reported by the U. S. Bureau of the Census for each of these years. In California 233 cases of the disease have been reported (1921-1941 by Beck). In California the infection is transmitted by the minute tick *Ornithodoros hermsi* (Wheeler) which is no larger than a bedbug. There is considerable variation in the conditions under which these 2 ticks are found in nature.

*O. turicata* transmitting the disease in Texas has been found especially in caves and overhanging ledges produced by water erosion on of river banks some of which have horizontal depth of only a few feet to perhaps 60 feet. They have a ceiling of clay sandstone 6 to 12 m. in height which may not be over 4 feet in height. The floor is covered with dry powdery dust or sand which may reach a depth of 5 inches. The entrance to some of the ledges may admit man and animals freely. Other caves have a horizontal depth of from 50 to 500 feet. The ceilings and walls are of sandstone and may be located several miles from a stream. Ticks located in the dust of the caves have an advantageous position for attachment to the legs of passing animal or of man. The caves located in proximity to rivers are sometimes subjected to flood water. The ticks are probably transported at times by such water to considerably distant places.

Francis (1939) has carried on laboratory observations extending over 7 years upon ticks collected from such caves in Texas in 1931 and has found that the spirochaetes of relapsing fever have survived within such ticks throughout a period of 6½ years. In 14 of the original ticks all females virulent spirochaetes were still present. He also found that these ticks harbored virulent *S. ochracea* cultures after 5 years of starvation and that a smelted relapsing fever to a monkey on which they fed. Davis (1943) reports that transovarial transmission of spirochaetes has taken place through five generations of *O. turicata* indicating that ticks kits if many months without spirochetal reservoir in the resident host.

The importance of these observations from the viewpoint of eradication of the disease is apparent since control measures must reckon with ticks hiding in caves and living in rodent burrows harboring virulent spirochaetes in their fasting bodies for 5 years and in their fed bodies for 6½ years (and probably longer) and transmitting the infection through the egg to the next generation of ticks.

(Some experiments with the African species *Ornithodoros moubati* have shown that this species when fed from time to time also will live as long as 5 years.)



In California the conditions of occurrence are different. There the disease occurs at elevations ranging from about 5000-8000 feet and especially in the region of lakes particularly Big Bear Lake in San Bernadino County and about Lake Tahoe in Placer and Eldorado Counties. Around both of these lakes there are a number of popular summer resorts and many privately owned summer cottages.

Wheeler (1938) has transmitted relapsing fever by the bites of infected ticks *O. hermsi* that had fed upon human cases of the disease to white mice and monkeys and other human subjects. Seven human subjects were exposed to the bites of infected ticks and one of them bitten by 2 adults developed the disease after an incubation period of 7 days and had 3 febrile attacks spirochaetes being found in the first 2 attacks. But of 7 rhesus monkeys exposed to the bites of 17 infected ticks only one monkey developed infection spirochaetes appearing after an incubation period of 16 days. Evidently susceptibility to infection varies greatly in human beings and monkeys. Wheeler also found it possible to transmit spirochaetes to white mice by the larvae hatched from eggs laid by infected ticks. However out of 672 larvae infested only 2 produced infection. Wheeler points out that this percentage is apparently sufficient to insure persistence of the infection. Clean larval ticks were fed on infected mice and later developmental stages of these ticks subsequently fed on normal mice when from 35 to 48 per cent of the ticks were found to transmit the infection.

Wheeler while conducting investigations in 1932 about Packer Lake California accidentally smeared his fingers with the blood from a chickaree or red tree squirrel (*Sciurus douglasii*) and there resulted a typical case of relapsing fever with blood smears positive for spirochaetes. Blood smears made later from these squirrels as well as from chipmunks (*Eutamias* sp.) proved positive for spirochaetes and subsequent laboratory inoculations of blood from these rodents into white mice were also positive for spirochaetes. Wynns and Beck (1935) have also given positive evidence that these animals constitute reservoirs for the causative organisms of relapsing fever.

It appears that this tick *O. hermsi* in California is typically a parasite of rodents and like other species of the same genus it feeds freely on a variety of experimental animals as well as on human beings. The female measures from 5-6 mm in length by 3-4 mm in width while the male is only slightly smaller. Therefore it is small as compared with other members of the genus.

Altitude appears to play a very important role in California, as the cases of relapsing fever have been confined to mountainous districts over 5000 ft. in elevation. The actual elevation however according to Wynns and Beck is of less significance than the climatic factors present in these particular locations. The wild rodents chipmunks and tamarack squirrel in which spirochaetes resembling *S. recurrentis* have been found are limited to the higher altitudes also which is evidence in favor of their acting as the animal reservoir of relapsing fever in California. On the other hand the species of tick incriminated is probably not limited to high altitudes. So in explanation of the peculiar distribution of endemic foci in this state the suggestion is offered that it is determined by the animal reservoir rather than by the transmitting agent. Altitude obviously does not play such a role in limiting the incidence of the disease in Panama and Texas. Hence the conditions under which relapsing fever occurs in California are very different from the epidemiological ones which exist in Texas and tropical America in both of which places ticks of the species *Ornithodoros* are very prevalent.

Very large numbers of ticks have been found in the native huts in Panama and in one survey 4880 specimens were obtained from 68 huts and certain caves in Denton County Texas have supplied ticks in abundance. In California however the ticks are found with great difficulty

and never in large numbers. If the ticks were more numerous in California and were brought into closer contact with individuals there would evidently be a higher incidence of the disease on account of the infection which is found in the wild rodents in California. The indications are that the ticks hibernate during the winter that they become infected in feeding on the rodent host and are disseminated in the spring at the time the rodents become active and leave their nests. Nicolle and Anderson from their tests upon relapsing fever in Tunis suggest the hypothesis that the blood spirochaetes of man were originally parasites of small burrowing mammals and that rodents have in the past commonly served as animal reservoirs of the disease.

**Other Ecological Conditions**—The prevalence of relapsing fever is also influenced by the different seasons of the year according to the favorable or unfavorable circumstances they furnish for the propagation of the intermediate hosts. In Southern Europe and North Africa the louse borne disease is usually a disease of winter and spring ending in the summer months. In North China also the incidence is lowest in the autumn and begins to rise in December reaching its climax in April and May. Naturally in the cooler winter and spring months the natives usually envelop themselves in thicker clothes and congregate together more for the sake of warmth. They are not liable to bathe in cold weather and lousiness becomes prevalent. The louse borne disease is uncommon in Equatorial Africa where particularly on account of the scantier clothing and high temperatures *Pediculus humanus* is unable to thrive. Also in the very hot months of spring and summer in India the lice become scanty and sometimes are destroyed by the high temperature. Cragg noted that in India if the temperature in May falls 10 or more degrees below the seasonal normal there may be a marked development of lice and epidemics of relapsing fever. This was true of the famine fever outbreaks of Bombay.

With the tick borne disease the seasonal incidence is different. Thus in California and Texas the occurrence of the cases runs practically parallel with the appearance and disappearance of the rodents concerned in the transmission. According to the dates of the onset the majority of the cases have occurred in June, July and August. An occasional case occurring in the winter might be due to infection from handling a rodent perhaps encountered or killed on a hunting trip during the winter. In those portions of Equatorial Africa where the seasonal variations are not great there is no marked seasonal incidence of the tick borne disease. Also a marked seasonal incidence has not been noted in the disease in the northern part of South America except that a somewhat greater incidence of it has been observed in the wet and rainy season when the native laborers and oil prospectors are more confined to their houses than during the drier seasons of the year and where the ticks are encountered especially at such seasons.

**Sex**—The disease is more common in males than in females. In a series of 337 cases studied by Chung and Chang (1939) in North China the sex distribution was 1 female to 17 males. Even after correction for

the ratio of admissions it was 1 female to 6 males. This ratio was especially probably due to the fact that most of the patients contracted the disease in army camps, poor houses, and small inns where the population was exclusively male.

In California where the disease is tick borne there have been twice as many males infected as females. With the louse borne disease in Europe the disease is also much more common in males than females and this is attributed generally to differences in the chances of exposure which are usually greater in males.

In a study of the disease in Changshu, Chang (1938) observed no cases in children below 9 years. He thought this was due to the fact that young children seldom crush the lice on the skin which is necessary to produce infection. However in Peking Chung and Chang (1939) observed 3 cases in children under 5 years of age but only 1 case in a child under 1 year. Young children in China would not be admitted to many of the public houses and institutions occupied by adults. Wynns and Beck (1935) found in California a tick borne disease in all ages from 4 years to above 55 represented among the infected cases.

#### PATHOLOGY

**Morbid Anatomy**—The skin is often jaundiced. Small haemorrhages may sometimes be noted in the nostrils, ears, stomach, intestine, uterus, or kidneys. The internal organs are not infrequently stained with bile. The spleen is usually enlarged and soft and often shows multiple infarctions. The liver may also be swollen. Parenchymatous degeneration of the liver, kidney and heart muscle may be present. The examination of sections of the spleen or liver often show the spirochaetes within endothelial leucocytes. In the spleen they may be especially prevalent and often particularly observed in miliary lesions in the malpighian bodies.

Griesinger (1857) pointed out that the peculiar infiltration of the malpighian bodies of the spleen was the most important point in the diagnosis of billious typhoid and its differentiation from yellow fever which so closely resembles it clinically. Lebert (1875) in Russia and Germany also described prominent malpighian bodies with small yellow foci and infarcts in the spleen.

Lubinoff (1884), Rabagliati (1905), Kulescha (1923), and others likewise noted miliary necrotic lesions in the spleen. Kritsch and Sideroff (1923) found spirochaetes especially numerous in such lesions. However, in a number of instances these lesions were of a pyogenic nature due to secondary infection with bacteria. Helen Russell (1932) who has studied the spleens of 15 fatal cases of relapsing fever in Africa has found in 11 of the cases in sections stained with haematoxylin-eosin miliary cell infiltrations of the malpighian bodies. In sections of 1 of the 15 stained by the silver method of Warthin-Starry spirochaetes were encountered. She believes that the spirochaetes may sometimes be found in the lesions of the spleen when they cannot be found in blood films made at autopsy and she concludes that the miliary lesions of the spleen which

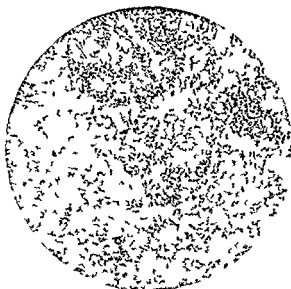


FIG 77a—Nucleus of a cell around Malpighian body. Stained with haematoxylin. (After H Russell. Court. y Roy Soc. Tr. p. Med. Hyg.)

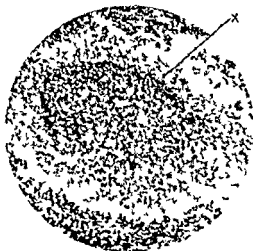


FIG 77b—Stained preparation of Warthin-Starry method. Black on the right is a single Malpighian body. (After H Russell. Court. y Roy Soc. Tr. p. Med. Hyg.)

consist of a zone of congestion and cell infiltration around the malpighian bodies are characteristic of relapsing fever. Spirochaetes are often seen in large numbers in these lesions in many instances breaking up in them. The miliary lesions can often be recognized macroscopically. In some instances the whole cut surface of the spleen appears dotted with enlarged rather transparent appearing malpighian bodies. Stained film preparations from the blood and from the brain may also show spirochaetes. Haemorrhagic meningitis has been reported in relapsing fever and Jähnel (1927) found spirochaetes in the parenchyma of 2 human brains. Numerous observers have reported their presence in the brains of rats and mice experimentally infected. The organisms frequently disappear shortly after the death of the host.

Chung and Chang (1939) in the study of 21 fatal cases found the majority of deaths were due to complications bronchitis pneumonia and bacteraemia. In only 4 cases was the relapsing fever infection apparently the sole cause of death. In 15 cases death occurred because of bacteraemia (6 with *Salmonella enteritidis* infection) or of pneumonia. 8 with broncho pneumonia and 3 with lobar pneumonia or both.

Ordman and Jones (1940) in South Africa where the mortality was about 9 per cent found the fatal cases associated with debility, heart failure pneumonia and intestinal infections.

#### SYMPTOMATOLOGY

**African Relapsing Fevers**—In African tick fever after a period of incubation of from 3 to 10 days the disease sets in rather suddenly with dizziness marked headache and general body pains. The temperature quickly rises to  $104^{\circ}$ – $105^{\circ}$ F or even higher and remains elevated during this primary febrile period except for slight morning remissions. Vomiting is a common symptom and may be bilious in character. Jaundice may occur and a miliary eruption or petechiae in the skin have been reported in some cases. Delirium may occur when the temperature is high. There may be rather marked praecordial oppression and a bronchial catarrh. The pulse in particular and the respiration in less degree are accelerated. Herpes and epistaxis may be noted. The bronchial manifestations seem to occur chiefly in the first febrile accession. The spleen is somewhat enlarged and tender but in many cases this is not noted. Spirochaetes are found in the peripheral circulation during the febrile accessions but not during the apyrexial intervals. There is great variation as to the abundance of spirochaetes. In some cases we may have to search several hundred fields before finding a single spirochaete. Severe cases may show them in abundance. A rather marked leucocytosis may be present in cases showing high fever and bronchitis. Albuminuria is frequently noted and rarely haematuria. After about 4 days the fever falls by crisis often below normal and possibly with great prostration and cardiac weakness.

A critical sweat is a feature of this rapid fall of temperature. During the afebrile period which lasts from 3 to 4 days to 8 to 10 days the

patient feels much better and his appetite and strength return. With the onset of the second pyrexial wave the severe symptoms of the first days are repeated as with the first febrile period. This second one terminates by crisis. Conjunctivitis is often present and iritis is not uncommon. Manson and Thornton have reported transient cranial nerve involvements coming on late in the course of the disease. The disease may be associated with uterine haemorrhages or abortion.

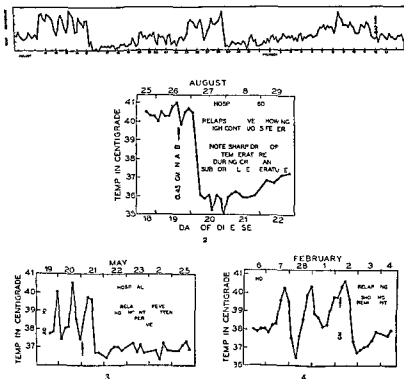


FIG 78.—1 Ch t f p rum t lly nf t d w th S u nt Pat nt t eat d w th novasenobenz 1 Sept 12 N fu th l p (C s of Chung a d W C urtesy Am J l T p M d)  
2 3 nd 4 show g th e dff nt typ f t m p tu u v m r l p ng f v (C of Chung a d Ch ng C urte y Ch n s Med J l)

In European relapsing fever the second febrile accession is usually shorter and of less severity than the first. Furthermore there are rarely more than 2 or 3 relapses. In tick fever however there may be as many as 10 of these febrile recurrences although there are usually only 4 or 5. In natives there is frequently only one febrile period this probably being due to an immunity resulting from previous infections.

**North African Type**—In the relapsing fever of North Africa which is usually louse transmitted the attacks are less severe and the number of

relapses rarely exceeds three. Sergeant (1938) in Algeria, finds that the relapsing fever there, due to the strain *S. hispanicum*, may be transmitted sometimes by the dog tick *Rhipicephalus sanguineus*. According to deBuen and Nicolle and Anderson the Tunisian form of the disease may be transmitted both by *Pedicularius humanus* and by a species of *Ornithodoros*.

A fever of Egypt formerly known as the bilious typhoid of Grewinger is now believed to be a form of relapsing fever. Clinically there may be marked bilious vomiting with great tenderness of the liver, enlarged spleen, later jaundice, albuminuria, haemorrhages into skin and internal organs, bone pains, especially about the knees and often a high death rate. The symptoms may at times suggest yellow fever but this disease has not been reported from Egypt. However on the West Coast of Africa cases with such symptoms might be mistaken for yellow fever. In the Sudan (1937) 374 cases and 48 deaths of the louse borne disease were reported while in Tanganyika where the disease is tick borne there were 1612 cases and only 17 deaths and in Uganda 453 cases with only 25 deaths indicating the greater severity of the louse borne infection.

Hawking examined the cerebrospinal fluid in 12 cases of East African Relapsing Fever (Tanganyika) due to *S. duttoni*. Five of the 12 gave rise to spirochaetes in mice on incubation. However spirochaetes were not found microscopically in the cerebrospinal fluids of the patients. Three of the patients showed symptoms of meningitis but in the other two there was no clinical evidence of this complication. General Biggam (1942) has found that in the Relapsing Fever (tick borne) in Tobruk and Palestine nervous complications were common and not infrequently ocular and facial nerve palsies were observed. Dixon (1943) has reported from Cyprus a case with Kernig's sign and paralysis of the arm and found marked urobilinuria in many cases.

**Indian Type**—In the relapsing fever of Asia there has been reported a marked tendency for the patient to collapse at the time of the crisis. There are rarely more than 2 relapses and in probably 25 per cent of the cases there is no relapse. From some of the Indian reports it would appear that there is a greater tendency to liver complications in the Asian types than elsewhere and such cases contribute particularly to the death rate from this disease. Bilious vomiting and jaundice, with a typhoid like state and the occurrence of various inflammatory complications especially parotiditis have been especially noted. The mind is usually clear but delirium may be present in severe cases.

Chung and Chang (1939) in a study of 337 cases of the disease in North China noted that in the cases without treatment the number of febrile attacks was from 3 to 5, the duration of which varied from 4 to 10 days with an average of 7 days. Splenomegaly was observed in 69 per cent and enlargement of the liver in 41 per cent. Jaundice occurred in 29 per cent and a haemorrhagic skin rash in 35 per cent with general glandular enlargement in 18 per cent. Some of the patients developed cerebral symptoms. Pneumonia occurred in 5 per cent and was a very serious complication accounting for 8 of the 21 deaths of the series. Only 60 per cent showed leucocytosis. Slight albuminuria occurred in 195 cases, of which 97 showed casts and 34 haematuria.

**Persian Type**—This relapsing fever is also known as Mianch disease and is generally somewhat milder than African tick fever or European relapsing fever. The febrile paroxysms are usually quite short but there may be 4 or 5 relapses. The spleen is not apt to be enlarged and jaundice rarely occurs. Spirochaetes are very scarce and thick film blood smears are sometimes necessary to detect them. Another form of relapsing fever frequently of even milder type has been described. Spanish type due to *S. hispanica*. On account of the mildness of this organism has been used as a source of infection in the production of relapsing fever in man.

venous inoculations of 2-3 cc of the blood in treating 230 cases of syphilis

**Relapsing Fever of Panama**—In 3 experimental cases produced by *O. talaje* the incubation period was 6, 11 and 15 days. The temperature of the first accession varied from 102 F to 104.5 F. Frontal headache and general body aches were the chief symptoms. Vomiting was noted in one case. The spleen was not enlarged. The first relapse was cut short in each case by arsphenamine.

**North American Cases**—Kemp and his associates found that the disease in North America resembles the European form in general with abrupt onset. In the majority of cases the febrile period lasted 3 days and varied from 2-5. Sudden termination of the fever by crisis was accompanied by profuse drenching sweats with a pungent odor. The relapses occurred at irregular intervals varying from 2-9 days. The pulse was accelerated in proportion to the fever. Polymorpholeucocytosis of mild degree was usual. A rash of rose colored spots was sometimes present on the trunk or limbs during the onset. A muscular asthenia of variable degree was the most common sequela. Haemorrhagic nephritis, iritis, cranial nerve paralysis and meningitis was noted in a small proportion of the cases. The spleen and liver were not enlarged in all cases.

### THE SYMPTOMS IN DETAIL

**The Temperature Curve**—This is the chief point in the clinical diagnosis of relapsing fever. The onset of the first febrile accession is abrupt and the temperature rapidly rises to 104 F or higher—sometimes 108 (Manson Bahr). After a continued high temperature for 3 or 4 days although at times only 24 to 36 hours the fever drops by crisis which is at times productive of collapse. Following an apyrexial period of 4 to 8 days we have a second febrile accession and there may be several of these wave like alternations of fever and apyrexia unless the patient is treated.

**The Nervous System**—Very marked frontal headache is a striking feature and the pains in back and limbs may be of great severity. In some cases the headache is more occipital. Cranial nerve involvement has been noted. There may be apathy but on the whole the mind is clear.

In a study of 1500 cases during the East African campaign J. K. Manson and Thornton observed 8 fulminating cases in which the onset was very sudden and in which the spirochaetes occurred in enormous numbers. Coma and death ensued in some within 24 hours. They considered that death might be brought about by the impaction of masses of spirochaetes in the cerebral capillaries.

Cawadias (1921) observed increased cerebro spinal fluid pressure and symptoms resembling meningo spinal encephalitis. Babes has observed haemorrhagic meningitis.

Pompano believes that the relapsing fever spirochaetes are remarkably neurotropic and he has found in guinea pigs the brain and cerebellum may be still infected 14 months after the primary inoculation. Mathis



and Durioux found that strains of spirochaetes isolated from the shrew in Dakar may persist in the brains of some inoculated mice up to 235 days. Many other authors have reported experimental evidence showing the involvement and persistence of the infection in the brains of infected animals as mice and rats.

As early as 1907 Soulie observed spirochaetes in the cerebrospinal fluid of a patient with relapsing fever with meningeal symptoms, and since this time spirochaetes have frequently been either observed in the spinal fluid or their presence demonstrated there by inoculation into animals. Thus Plaut and Steiner found that in patients suffering from general paralysis of the insane who had been infected for treatment with the African variety of relapsing fever the cerebro spinal fluid in 5 of 10 cases was capable of producing infection (relapsing fever) in animals and the fluid remained infectious in one instance as long as 69 days after the original infection and when the patient's blood was no longer infective. Lodegysky (1938) has studied the cerebro spinal fluid in 27 cases of African relapsing fever (*S. duttoni*). In 8 cases the fluid was normal but 2 of these showed changes later. The other 19 all showed a leucocytosis usually an increase of lymphocytes. In 3 cases spirochaetes were found by direct examination. Meningeal symptoms were frequent. An increase in pressure was observed in 15 cases and lumbar puncture, by relieving the pressure was usually followed by an improvement in the condition.

Chung (1938) examined the cerebro spinal fluid in 26 patients in North China. In some instances the physical properties of the fluid were found to be normal but in many instances there was a definite increase in the leucocytes chiefly in the lymphocytes and in a few instances the pressure of the fluid was somewhat increased. Of 16 patients in whom the cerebrospinal fluid was subjected to the Wassermann test, from 4 times 9 showed a transient but clear cut positive reaction which suddenly became completely negative 1 to 3 weeks later. The cerebro spinal fluid from 6 cases was examined by dark field but no spirochaetes were seen. However when the fluid was inoculated into 7 squirrels 5 developed relapsing fever.

**The Digestive System**—Anorexia and vomiting are features of the febrile periods which usually cease in the fever free periods. In some types bilious vomiting may be marked.

**The Circulatory and Respiratory System**—The pulse rate is much accelerated and there may be some praecordial distress. A bronchial catarrh is frequently present in the first febrile paroxysm. In some epidemics pneumonia has occurred in 5 per cent and has proved to be a serious complication.

**The Liver and Spleen**—Splenic tenderness and moderate enlargement are fairly constant features. The liver may suffer severely in the so called bilious typhoid and marked jaundice may ensue with a typhoid state.

**The Genito urinary System**—In about two thirds of the cases more or less albuminuria is present in about one third casts are found and in a small percentage haematuria.

Chang (1938) found that 27 (26.9 per cent) showed spirochaetes in the urine, most of which were dead or motionless though occasionally living ones were observed. One of 6 squirrels inoculated with urinary sediment developed relapsing fever. Whenever there was considerable haematuria during the febrile period more spirochaetes were likely to be present in the urine than otherwise. Viable and virulent spirochaetes were recovered from the prostatic fluid of one patient.

In pregnant women haemorrhages from the uterus are not uncommon and abortion usually occurs.

*The blood examination* is the most important procedure for diagnosis. The spirochaetes, which are usually only found in the peripheral circulation during fever periods, are often not so numerous in tick-borne relapsing fevers as in the European forms. When spirochaetes are scarce it is more satisfactory to examine Romanowsky stained specimens especially with the Giemsa staining. Spirochaetes sometimes may be found during the apyrexial period by using thick films or by inoculating mice. The spirochaetes show a varying number of undulations. There may be a curving of the end or the spirochaete may assume a ring form. These forms are more common towards the end of the paroxysm and then we may find 2 or more clumped together. The disease when severe shows a well marked polymorphonuclear leucocytosis with at times an increase of large mononuclears. This latter however may be connected with a concurrent malaria or amoebiasis.

**Wassermann Reaction**—Fairley (1936) reported a strong positive complement deviation test in about 25 per cent of the cases of relapsing fever both during the pyrexial stage and in the apyretic periods between the early relapse. Korshum and Liebtied (1909) performed a single Wassermann reaction on each of 50 patients in different stages of relapsing fever and found 28 positive. Fairley and Sullivan (1919) examined 32 patients with relapsing fever in Cairo and found a positive reaction in 10 per cent during the febrile period and in about 7 per cent during the afebrile period.

Roaf (1922) concluded that a transient Wassermann reaction might be found to be a constant phenomenon during the acute stage of relapsing fever. He thought the transient character of the reaction might distinguish it from a reaction due to syphilitic infection. Gillespie (1935) in reporting the cases in the United States also noted a transitory positive Wassermann reaction. On the other hand Brynogle (1927) and others have reported negative reactions and Tsun and Chang (1936) false positive reactions in about 8 per cent. Chang (1938) found in 28 cases in China that 11 showed a positive Wassermann reaction though on reexamination of 6 later 5 had become negative. Chang (as noted) found in 16 patients whose cerebrospinal fluid was subjected to the Wassermann test from 1 to 4 times 9 showed a transient but clear-cut positive reaction which suddenly became completely negative 1 to 3 weeks later.

**Treatment of Syphilis**—Infection with relapsing fever has been suggested and tried as a therapeutic measure for cerebral syphilis as has malaria. This treatment has been employed recently in Germany and Austria, Northern Africa, the United States and China. Infection has been transmitted by direct intravenous injection of the blood containing the spirochaetes (d'Ayala 1931) by infected lice (Chung and Wei 1938).

or by infected ticks (Wheeler, 1938) Although beneficial results have been reported it is not clear that such results are superior to or as favorable as those reported with malaria treatment

Mas d Ayala (1931) on account of the mildness of the fever produced by the strain with which he worked *S. hispanica* reported 230 cases treated by intravenous inoculation of 2-3 cc. of blood taken from a relapsing fever patient during the febrile period Chang (1939) in 3 patients who contracted relapsing fever through transfusions of blood from patients (without relapsing fever symptoms at the time) found the incubation periods were 4-6-4 days Chang has observed 11 cases in which the recipients of blood transfusions developed relapsing fever following one or several transfusions In these instances the blood had been taken from donors either in the incubation or remission periods of the infection and when they had no unfavorable symptoms

Pampana noted that the relapsing fever spirochaetes were remarkably neurotropic in infected guinea pigs and as noted he found in some instances the brains were still infective 14 months after primary inoculation Mathis and Durieux found that strains originally isolated from rodents in Dakar persisted in the brains of some sub inoculated mice after 235 days

Wheeler (1938) employed the tick *O. hermsi* infected with relapsing fever spirochaetes and transmitted the infection to 6 men afflicted with cerebral syphilis They were subjected to the bites of several infected ticks Only 1 of the patients became infected after an incubation period of 7 days These tests and other tests on monkeys showed that not all persons are equally susceptible or capable of infection with the disease by this tick It is interesting to note that in the positive case the patient had received a systematic treatment for syphilis of neosalvarsan and bismuth for nearly 17 months prior to the feeding of the infected ticks upon him

Chung and Wei (1938) conveyed the infection to 6 volunteers and 4 patients with general paralysis of the insane by the louse *Pediculus humanus* The incubation period by the cutaneous route was found to be about 11 days

### PROGNOSIS

The mortality has been usually reported as about 2 to 5 per cent with the exception of the very serious form in which jaundice is present when the death rate may exceed 50 per cent as in some of the West African epidemics A high mortality is more often observed in patients who have suffered from malnutrition and who are otherwise feeble and old Chung and Chang (1939) in a study of 337 cases, mostly of the poorer classes in Peking had a mortality of 6.2 per cent while Gillespie (1935) who summarized several hundred cases in the United States noted no fatal cases A serious feature of the disease is the length of its course which sometimes extends from 6 weeks to 2 months Since salvarsan and neosalvarsan have been found to be specific in the treatment of the disease the mortality has been reduced to low figures as in the United States

### DIAGNOSIS

The disease most likely to be confused with relapsing fever is malaria and for this differentiation the finding of the parasites of either disease is of first importance

Dengue may be suspected but the leukopenia, lack of splenic tenderness, lack of tendency to vomiting and presence of post orbital pains may be of assistance in differentiation As there is a leucocytosis in both

relapsing fever and smallpox and similar headache and backache confusion might exist were the parasites not found and before the eruption of variola appears

Yellow fever has many features in common with the bilious type of relapsing fever but there is no leucocytosis in yellow fever and there is no characteristic albuminuria and slow pulse in relapsing fever Influenza may sometimes be confused with relapsing fever in its early stages

In a case of relapsing fever with jaundice confusion might arise with Weil's disease inasmuch as a blood smear might show spirochaete like organisms somewhat resembling those of relapsing fever

Typhus fever shows a less abrupt onset and the marked mental symptoms (stupor) and dark macular eruptions about the trunk on the 4th to 6th day may be of aid in differentiation Also the Weiz Felix reaction does not occur in relapsing fever

**Laboratory Diagnosis**—During the febrile phase of the disease the spirochaetes frequently may be demonstrated in films of blood stained by one of the ordinary Romanowsky stains or by dilute carbol fuchsin or they may be seen in fresh preparations examined by dark field illumination They occasionally may be found in the afebrile period When not numerous thick films should be prepared for examination If they are not found in films a mouse should be inoculated with the blood Within 24 or 48 hours the spirochaetes may be found in the blood of the mouse if they were present in the inoculum For demonstrating them in tissue sections silver impregnation methods are used Lowenthal's reaction is sometimes of interest when the case is first seen in the apyrexial period and no spirochaetes are visible in the blood The reaction consists of adding to a drop of blood from a case showing spirochaetes a drop of blood from the patient suspected of having the disease After incubation for 30 minutes the spirochaetes lose motility and become agglutinated if the case is one of relapsing fever If the patient is first seen during the apyrexial period if lice or ticks are collected from him and examined spirochaetes may sometimes be found in them There is usually a well marked polymorphonuclear leucocytosis in acute cases \*

#### PROPHYLAXIS AND TREATMENT

*Prophylaxis* depends especially upon the avoidance of places and contact with individuals infested with ticks bedbugs and lice In Africa and Central America the habitations of the natives where infested ticks may hide themselves in cracks in floors and walls are to be especially avoided As the tick *Ornithodoros* feeds at night a night light is of value as a repellent Destruction of the spirochaetes by salvarsan injection is important prophylactically as well as therapeutically—the reservoir of infection for lice or ticks being eliminated

In the louse transmitted forms prophylactic measures should always be aimed at the destruction of lice and their eggs and it should be borne in mind that both the head louse and the body louse can transmit the disease Hence delousing of individuals and of their clothing and heads

Stein (1943) prepares an antigen by taking 1 c.c. blood with saponin and centrifuging for complement fixation agglutination test

is important. The destruction of ticks particularly of *Ornithodoros moubata* is difficult except by burning. They sometimes apparently remain uninjured after being placed in solutions of cresol and they will also live for years without feeding. (See Francis and Davis p 341.)

**Prophylactic Inoculation**—A few attempts have been made to immunize the inhabitants of an infected district in Russia by inoculations of killed cultures of *S. recurrentis*. However Sergeant (1938) has found that attempts at vaccination in animals by means of dead spirochaetes killed either by cold or exposure to bile gave negative results.

**Treatment**—Arsphenamine preparations are usually regarded as specific but a few cases do not respond satisfactorily to treatment. The preparation should be given intravenously. Manson Bahr (1935) recommends salvarsan in doses of 0.3 gm to 0.9 gm according to the age of the patient and severity of the case the dosage being reckoned as 0.01 gm for each kilogram of body weight. St John (1937) states that in fully 80 per cent of the cases the intravenous injection of 0.3 gm of salvarsan or 0.45 gm of neosalvarsan will end the infection. He recommends this smaller than the usual therapeutic dosage pointing out that relapsing fever infection lowers the resistance of the individual to the toxic properties of the drug. Gillespie (1935) recommends a single intravenous injection of 0.01 gm of neoarsphenamine for each kilogram of body weight administered at the onset of the paroxysm which he believes will effect a cure in practically every case in the United States. He believes that there is a close correlation between inadequate doses and relapses often with severe complications. It is usually recommended that the drug is most efficacious when given in the early stages when the temperature is rising. It ought not to be given when the crisis is imminent as then a grave reaction may occur due probably to the great destruction of the spirochaetes and the liberation of their toxins with corresponding aggravation of the symptoms and fatal collapse may result. Manson Bahr recommends if it is not given in the first attack one should wait until the first relapse and then give it on the rise of temperature. If a relapse occurs a second injection may be given. After the injection of arsphenamine the symptoms are often aggravated for a short time.

Chung and Chang (1939) have found the most annoying symptom following the injection of neoarsphenamine was vomiting which was severe in 15 of their cases. The commonest reaction however was an increase in the fever and headache. The temperature was frequently raised 1 or 2 degrees and in a small percentage of the cases from 2 to 4½ degrees. In 3 cases it rose 4 to 6 degrees Centigrade and 2 of these cases resulted fatally. In their series of 281 treated cases 16 patients relapsed and required a second specific treatment. In general the great majority of the cases occurring in otherwise healthy individuals recover without any specific treatment at all though on account of the relapses convalescence is often prolonged.

Manson Bahr states that albuminuria generally does not constitute a contraindication to salvarsan treatment. Some observers have not found

treatment with neoarsphenamine satisfactory. Avanesov (1938) in Afghanistan and Delphy and Rafy (1939) in Teheran found that while in some the treatment was effective other cases proved refractory to it. Francis (1939) reports 3 cases in the United States unsuccessfully treated with neoarsphenamine. One of the cases received 0.5 gm of neoarsphenamine at each of 4 relapses and recovered without further relapses. Another case received 0.45 gm of neoarsphenamine immediately after the onset fever had terminated and at each of the first 2 relapses but he had 3 more relapses untreated with neoarsphenamine. In a case which resisted treatment with neoarsphenamine Francis employed deep injections into the buttock of bismuth preparations the first 4 injections being with bismuth salicylate in oil 0.13 gm and the last 10 being with thio bismol 3 gr each. He also received serum intravenously taken from a convalescent relapsing fever patient in doses of 40, 40 and 20 cc at the beginning of his 9th relapse which was followed by recovery. Plaut and Steiner also found neosalvarsan unsuccessful in treating some cases of relapsing fever in cerebral syphilitics who had been infected as a therapeutic measure. Todd (1930) has also found neoarsphenamine less effective in controlling relapsing fever in Africa and recommends the intramuscular injection of 0.2 gm of sodium potassium bismuth tartrate dissolved in 2 cc of sterile water for an adult this dose being repeated on the following day.

German investigators have recommended two gold compounds solganol B and A 69 the former of which contains 36.5 per cent of gold. It has been claimed that residual infections of the central nervous system were eliminated by these preparations. Hawking (1939) has found that on spirochaetes *in vitro* solganol had little action.

Sergeant (1938) found novarsenobenzol to have no curative effect in animals except in toxic doses against the strain *S. hispanica* in Algeria. He found however in the treatment of infected guinea pigs that the serum of refractory animals or the serum of convalescent individuals was effective.

Mapharsen has been used successfully in a few cases and it has been especially recommended for treatment in the United States Army. It should be given in doses of 0.04 to 0.06 gm.

Heilman and Herrell (1944) have studied the effectiveness of penicillin in relapsing fever. The penicillin produced in mice by a single strain named B 11 N 3. Although 500 Oxford units of penicillin per cubic centimeter did not cause a visible decrease in motility of the spirochaete in 10 for 7 hours the motility ceased later in both treated and control tubes. Inoculations in heavily infected mice used the complete absence of the spirochaete in the blood smears in 2 or 3 days. Of 6 treated mice only 1 (1 per cent) died. The untreated mice which survived exhibited relapses. Only 4 of the treated mice had a relapse. Augustine, Weinman and McAllister (1944) injected rats infected with the strain (O 3) with penicillin large dose being employed. At the end of 7 hours no spirochaetes were visible in the treated mice but were still present in the blood of the control animals. Eagle and Magnuson (May 1944) in further experiments on rats and mice showed that doses of 130,000 and 100,000 units per kilogram were necessary to cure the half-th infected rats and mice 400,000 units per kilogram were necessary to cure more than 95 per cent of the animals and this was one half the dose which killed a significant proportion of the rats. Hence the therapeutic use of the drug for the treatment of this infection in man would probably not be warranted.

Careful nursing liquid diet with plenty of water, and cold sponging when the temperature is high must be maintained until after the crisis together with treatment of any special symptoms which may arise. The headache is often relieved by an ice cap and the general pain alleviated by aspirin. During the crisis digitalis morphine or caffeine may be indicated to support the action of the heart. Adrenalin given hypodermically every four hours is sometimes valuable in cases with collapse.

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## Chapter VIII

# INFECTIOUS JAUNDICE

## (*Leptospiral Jaundice*)

### SYNONYMS AND DEFINITION

**Synonyms**—Leptospiral jaundice spirochaetal jaundice epidemic jaundice Spirochaetosis icterohaemorrhagica typhu bilieux Weil's disease

**Definition**—Infectious jaundice is a febrile spirochaetal infection caused by *Leptospira icterohaemorrhagiae*. In various parts of the world this organism is common in rats which constitute a natural reservoir and a source of human infection. The disease is characterized by a sudden onset with rather high fever headache and vomiting. Jaundice which is common usually appears about the third or fourth day when the urine shows albumin and bile. Haemorrhages especially epistaxis are common and the liver and sometimes the spleen show enlargement. Ictynucleosis is present.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History**—The form of jaundice noted in the forces of Napoleon during the Egyptian campaign was probably infectious jaundice. It was first recognized as a distinct disease by Weil in 1886 who described it as an acute infectious disease characterized by jaundice swelling of the spleen and nephritis. Inada and his Japanese colleagues discovered the causative spirochaete in 1915 and later noted its frequency in rats and in 1917 infected guinea pigs with the organism from some of these apparently healthy rats. Sporadic cases of leptospiral jaundice were observed during the World War along the Western Front among the French British Italian and German troops none however in the American Army. It was also reported among the troops in Gallipoli.

**Geographical Distribution**—It has been common in Japan and Egypt and is also endemic along the North African coast and the shores of the Mediterranean in West Africa and the Congo. Cases have been observed clinically in the Sudan. However Kirk (1938) does not think it is prevalent there since he examined 259 rats in the north Sudan and found none infected and he points out that the spirochaete of Weil's disease has not been detected in the Sudan case. Alston and Brown in 1937 were unable to find it in Egypt Arabia and Persia.

During the Russo-Turkish war (1877) cases of jaundice seen in the Balkans by Sandwich were probably infectious jaundice as the disease was frequently noted in that region during the World War. In addition

to the cases which occurred among the troops in Flanders and in Italy outbreaks were also reported among the British troops in Gallipoli and in Egypt. The cases of the disease which the writer observed on the Western Front were of a milder character than of the type described in Japan.

In Europe cases have been observed recently especially in Holland Sweden Germany France, Czechoslovakia Italy Eastern Europe Spain Portugal Greece Albania and Russia.

Bessemans (1939) does not think the disease is common in Belgium as he was only able to find 2 sera in man which would agglutinate the leptospira. Van de Walle (1939) examined 100 dogs in Antwerp and found *L. schlerohaemorrhagiae* in 15 and *L. canicola* in 29. However he states human infection with *L. canicola* of dogs is unknown in Belgium.

In recent years sporadic cases have been reported in London and Scotland and outbreaks of the disease have been described in coal miners in Scotland by Buchanan (1934) in Wales by Sladden (1939) and Northeast England by Swan (1938) in sewer workers of London Liverpool and Glasgow by Fairley (1934) and Stuart (1938) in fish workers of Aberdeen by Davidson (1934) and in tripe workers in Glasgow by Stuart (1938).

In the United States the disease has apparently been comparatively rare. Glotzer (1938) who reported in New York the second case in fish workers in the United States was able to find 20 other cases in the literature which had been diagnosed since 1922 by bacteriological examinations in this country. Cases have been reported in New Jersey Pennsylvania and California. Blake (1940) has recorded a case from Connecticut in which diagnosis was made by a positive agglutination test. Havens Bucher and Reimann (1941) report from Philadelphia an outbreak in 7 young men who bathed in a stream probably polluted by infected rats and contracted an acute infectious disease in different grades of severity. Five were affected mildly and 2 were severely sick, 1 of whom died. Studies of the fatal case and serologic evidence in the other severe case proved the diagnosis of *Leptospira icterohaemorrhagica*. The blood of the fatal case was injected into guinea pigs that died of a fatal infection and the organism was found in sections of the liver and kidneys.

Packham (1937) mentions 14 instances of infection not published. He has studied the reports of many epidemics in the United States of epidemic jaundice but in most of the cases there was only clinical evidence of the nature of the disease. A large number of the cases of epidemic jaundice occurring in the United States evidently have another origin as has been demonstrated by the investigations of Blumer (1923) Pickles (1936) Norton (1939) and others. It therefore would perhaps be preferable to employ the term leptospiral or spirochaetal jaundice for the form we are describing in this article. Outbreaks of jaundice have also been reported not only in the United States but in Norway and Sweden and England (1934) in which no evidence of a leptospiral nature has been obtainable and in which there was no evidence of leptospira in the blood or urine and no evidence of immune bodies in the serum of the patients.

In the Far East recent reports of leptospiral jaundice with bacteriological diagnosis have been made particularly in the Dutch East Indies Borneo and the Celebes Cochin China Indo-China the Andaman

Islands and the Malay States India Japan and China and Australia While in the Western Hemisphere cases have been reported in the French West Indies Guadeloupe and Martinique (1938) and Peru and Brazil (1939) and French Guiana (1940)

In all probability the geographical distribution given above is not complete and will be extended from time to time For example the disease has been presumed to exist on clinical grounds for a long time in India and McRobert (1934) had found it to be common in Burma However apparently the first case reported in India in which the leptospira was demonstrated was by Das Gupta and Chopra in 1937 In 1938 Das Gupta reported 5 cases 3 fatal in Calcutta 6 cases having been discovered in 8 months In 1941 he diagnosed cases in Assam With the increased number of laboratory examinations being made for the diagnosis in various parts of the world cases will doubtless be discovered elsewhere For as at least several species of rats and mice harbor the parasite and act as reservoirs we may expect the human disease to occur in every part of the world where hygienic habits do not prevail and where these rodents come into close contact with man and contaminate the water and food especially by their urine containing the leptospira

### EPIDEMIOLOGY AND ENDEMOLOGY

In Europe the disease occurs chiefly in the late summer and early autumn months July to October In Holland while Schuffner found



FIG 79

FIG 79—Showing *L. plospira* (After N. Guichon, Journal of Experimental Medicine)



FIG 80

FIG 80—A group of *L. plospira* (After N. Guichon, Journal of Experimental Medicine)

that cases might occur in any month it prevailed especially from July to October in the years when it showed an epidemic exacerbation He thought this the result of infection particularly among bathers and swimmers in the warm months The incidence of the disease was greater in southern Holland where there is low salinity of the water compared with the much lower incidence in northern Holland with higher salinity Ruys has shown that the spirochaetes survive longer in waters with a low degree of salinity

In Japan it has been reported more commonly in the autumn months September to November In the Andaman Islands the cases prevailed during the period of the southwest monsoon occurring in males who are engaged particularly in outdoor occupations at that season

**Manner of Infection**—Human infection may result from the ingestion of food or water contaminated with the urine of infected rats The leptospirae occur in the urine kidneys and faeces of wild rodents especially *Mus norvegicus* *Mus alexandrinus* *Mus rattus* the mouse *Mus musculus* and the vole of Japan *Microtus montebellii* Also in the bandi

coots, *Nesokia bandicota* *N. bengalensis*, in the Indies, as well as in the dog and the fox. In Europe Schüffner (1934) believes the sewer rat *Mus norvegicus*, is especially concerned. In Malaya Fletcher (1934) found the prevailing *Mus rattus* frequently infected. The percentage of rodents infected with leptospirae varies greatly in different localities. In the United States in different examinations from 4-33 per cent of infection has been found. The results of a number of surveys in different parts of the world have been as follows

Meyer (1938) in California	Positive 33%
Schüffner (1934) in Holland	Positive 56%
Zimmerman in Amsterdam	Positive 40%
Vasilevsky (1933) at Kiev	Positive 30%
Tartaglia (1939) at Dalmatia	Positive 20%
Stevenson (1922) in London	Positive 4 30%
Middleton (1929) at Oxford	Positive 41%
Pawan (1931) in Trinidad	Positive 25%
Cotter and Pavers (1935) in Queensland	Positive 30%
Ido in Japan	Positive 53%
Das Gupta (1938) in Calcutta	Positive 10%

Schuffner (1934) found all the local epidemics he studied were usually accompanied by heavy murine infections up to 56 per cent. He considers that human infections are the result of the contamination of the water by the urine of the infected rats. The percentage of infection among older rats was found to be always greater than in the young rats under 20 cm. In the younger animals the infection might be 3 per cent while in the adults up to 45 per cent. It has been suggested that the disease was primarily epizootic in wild rats but that these rodents have become tolerant and that some of them may harbor the parasite throughout life. It is regarded probable that the infection may be passed from one rat to another by way of food polluted with urine and by sexual intercourse. Uhlenhuth and Zimmerman (1933) found it was possible for white rats to become infected when placed in cages with infected wild rats. Human infection may also occur from the bites of rats. Uhlenhuth has reported a case in a laboratory attendant who was bitten by an infected white rat and subsequently developed Weil's disease. Blumenberg (1937) reports 3 cases of Weil's disease one fatal among laboratory attendants in which the infection was almost certainly acquired from handling rats. Infection may also occur in cages from infected rats to healthy guinea pigs through direct contact.

In some localities it is thought that dogs may constitute a reservoir of human infection. Schüffner found only one prevailing strain of *Leptospira icterohaemorrhagiae* in man in Holland. However in the examination of 50 dogs in which the infection was fatal in 24, were found to be infected with a typical Weil strain and 28 with a different strain which he named *L. canicola*. The latter strain which he cultivated from dogs produced no jaundice. It was not particularly pathogenic for guinea pigs and it differed serologically from *L. icterohaemorrhagiae*. Later he found one human case also without jaundice and this patient was infected with this dog strain *L. canicola*. Van den Walle (1938) examined the blood of 100 dogs in Antwerp and found *L. icterohaemorrhagiae*.

haemorrhagic in 15 and *L. canicola* in 20. Meyer, Eddie and Anderson Stewart (1938) in a study of a very fatal disease in dogs in California in 67 autopsies found 33 of the icteric type corresponding to the classical Weil's disease and 36 of the haemorrhagic type resembling the Stuttgart disease. In a study of many cultures leptospirae were found in 6 dogs which were definitely jaundiced. This strain was identified by Schüffner as *L. canicola*. However in its power to produce icterus the Californian strain thus differs from the Dutch strain. A veterinarian infected with the Californian dog strain showed a definite icterus and nephritis and his serum 8 months after recovery agglutinated *L. canicola* in a dilution of 1:300 and *L. terohaemorrhagiae* a dilution of 1:30. Twenty-two strains isolated by guinea-pigs all produced fatal infections with jaundice after 2 or 3 passages. Meyer points out that *L. canicola* was not found in the rat population so that the host relationship of the leptospirae in California is apparently the same as in Holland, rats being the source of typical Weil's disease due to *L. typhosa* and dogs the reservoir for *L. canicola*. Kowenaar and Wolf (1930) in Sumatra thought that dogs might constitute a reservoir of infection as they found 6 per cent of the normal dogs in Medan infected. A form of leptospirosis known as the yellows has also been encountered in foxhounds. Catchpole (1934) has noted an epizootic in young silver foxes. The older animals had apparently become immune. The infection has also been reported in leopards.

In the rare case of human disease in which the infection has been acquired from contact with the dog *L. canicola* has been the infecting organism. Jaundice has not been noted in Europe in this form of human disease though as mentioned above Meyer found jaundice in a percentage of his dogs and in one human case. Schüffner states he has never found the dog strain in sewer rats and has been unable to infect white rats with it. Pester (1938) records the infectious gastritis of dogs (Stuttgart disease) as identical with Weil's disease. He is of the opinion that *L. typhosa*, *L. icterohaemorrhagiae* and *L. grippityphosa* have a similar antigenic structure but show many variants. He found however that *L. hebdomadis* shows marked differences and he regards it as a different species. Although *L. typhosa* and *L. grippityphosa* had been identified by serological tests as present in infections of dogs in the United States Colonel Ray and Randall (personal communication 1944) obtained the first actual isolation of *L. typhosa* from the blood of a dog. The blood of this dog was inoculated into a golden hamster which died of typical *L. typhosa* infection.

Pandall (Feb. 24 Aug. 1943) isolated 2 strains of *L. typhosa* from dogs. In both instances the owners (2) of the dogs also became infected and *L. canicola* was isolated from the urine of the dogs. Several hamsters inoculated from the dogs died with typical leptospirosis. Thus fatal infections in hamsters were produced by both *L. canicola* and *L. typhosa*.

Morton (1942) hidshow that *L. icterohaemorrhagiae* killed 3 to 4-week-old Syrian hamsters in 5 to 8 days with typical icterus. Using a single strain of *L. canicola* he found hamsters survived the injection of this organism although gamma-rays could be detected in the blood stream. However Lars (April 1944) has found as has Randall that hamsters (*C. crinitus*) are susceptible to infection with *L. typhosa* and *L. icterohaemorrhagiae* both causing fatal infection. No other convenient animal has been shown to develop lesions or to succumb readily when infected with *L. canicola*.

The leptospirae may gain entrance to man through the skin and mucous membranes, the conjunctiva, mouth and intestines.

In outbreaks of the disease it has sometimes been possible to distinguish among the population certain groups of people more disposed to infection than others and the disease has been discussed as an occupational hazard. Thus it often occurs in people whose occupation leads them near water, like barge men, wharf men, fish workers, slaughterhouse employees, in fact those who carry on their work in localities infested with rats. Davidson and his associates (1936) reported an outbreak of 40 cases in Aberdeen, Scotland, chiefly among fish workers who were employed in handling fish.

in rat infested premises, the floors of which were covered with slime and offal. Abrasions of the skin frequently occurred in such work thus affording greater opportunity for infection. Guinea pigs were infected with water obtained from this slime and developed Weil's disease. Two similar cases in fish workers in the United States have been reported and 10 cases have been reported in tripe workers in Glasgow (Stuart, 1939).

Also cases of infection have frequently been observed in workers in mines especially in wet mines, as reported in Japan by Ido, Buchanan (1934) in Scotland and in Aberdeen and elsewhere. Buchanan, in connection with the outbreak of Weil's disease in the Scottish mines isolated *L. icterohaemorrhagiae* from the slime in one of the mines and produced the disease in guinea pigs by inoculation. Allston and Fairley (1934) have shown that the disease is not uncommon among sewer workers in London and Liverpool.

The disease is also sometimes encountered among harvesters, farmers, sugar cane cutters, etc., after prolonged rainfall, as reported in an outbreak in Queensland in 1935 by Cotter and Johnson (1938) and in Dalmatia by Partagha (1939) in both agriculturalists and fisherman.

Trench warfare, with wet conditions producing sodden conditions of the skin and rats furnished conditions favorable for infection in 1917 in France. However, the cases that the writer saw there, as noted, were of a much milder character than most of those described from Japan and did not resemble yellow fever clinically sufficiently even to suggest the diagnosis of the latter disease. Indeed the mortality from the disease on the Western Front was low, not more than 4 or 5 per cent. In Japan however, the mortality has been as high as 32 per cent. Nevertheless Noguchi later reported that the organisms sent to him from some of these cases in European soldiers were identical with the Japanese strain *L. icterohaemorrhagiae*.

Infection has followed accidental falling into canals contaminated with human or animal refuse or in persons who have attempted to commit suicide by jumping into such water where the banks are likely to harbor rats which carry the leptospirae and infect the water with their urine. In Holland Schuffner (1934) reported 37 cases of water accidents following which Weil's disease developed. Naftalin, (1938) records a case of Weil's disease in a man in England who fell into a canal in a motor car accident and swallowed a good deal of canal water. After 5 days he developed pains in the legs and became ill with fever. He died 20 days after the accident. Postmortem examination revealed typical Weil's disease and *L. icterohaemorrhagiae* in the liver, kidney and spleen but not in the lungs. Two other passengers in the car were also half drowned but made uneventful recoveries.

Jorge (1931) reported an epidemic in Lisbon in which a leptospira was isolated from a public fountain. Infection may also occur in those who swim and bathe in contaminated water. Romijn (1932) reported 34 cases traced to a swimming tank located in one of the ancient city moats, a narrow canal with stagnant water. The water was changed by dis-

charging it into the river and letting in a fresh supply from the same source but the water was exposed to constant pollution by the adjoining human dwellings and as a consequence the area was teeming with rats. Epidemics have occurred elsewhere as in Germany Italy and the Belgian Congo after bathing in river pools. In Germany the name slime fever has been given to a form of Weil's disease said to be acquired from bathing. Seven hundred cases were reported in 1936. In these cases the attack began with a rigor and a rise of temperature in some cases to 104 F. and infection through the conjunctival sac was observed in some of the cases.

In eastern Europe Korthof and Tarrasoff (1934) have described under the name of swamp fever *maladie de la vase* or marsh fever a form of Weil's disease without jaundice which occurs in epidemics in the workers in these swamps. Tarassoff has isolated from the swampy water a leptospira which on inoculation into guinea pigs appeared to be identical with the strain isolated from the human cases and to which he gave the name *L. grippo typhosa*.

The infection of water appears to be in no way proportional with the degree of visible contamination. One may fall into very foul looking water without developing Weil's disease and in some of the swimming pools proved infective the water was normal in appearance.

The hydrogen ion content of the water and its salinity apparently affect the time that the leptospira will remain virulent in it. Van Thiel (1937) found that during the summer season of warmer weather *L. ictero haemorrhagiae* survived for at least 22 days after infection of the water and that its virulence remained intact at least as long as that. He points out that while epidemiological data do not indicate that human beings become infected from *L. canicola* by bathing this is nevertheless possible under certain conditions.

In addition to the strains of leptospira already described in man other saprophytic strains have been studied as *L. biflexor* which was demonstrated by Wolbach and Bingham (1914) to be nonpathogenic. Uhlenhuth and Zuelzer (1923) isolated by culture from aqueduct water a spirochaete which subsequently after cultivation in blood serum acquired distinctly pathogenic properties for animals. This spirochaete in doses of from 2 to 4 cc. of the culture when injected intraperitoneally finally after several passages produced in guinea pigs a disease which after 4 to 8 days caused death. The entire appearance of the animal so infected corresponded with that of Weil's disease. This organism also corresponded in different serological reactions with *Leptospira icterohaemorrhagiae*. Karshner however did not confirm this work and this study has been interpreted differently. Schüffner suggested that the guinea pigs might have become infected from contact with rats. Schüffner (1934) has shown that the nonpathogenic *L. biflexor* may occur in almost any water and that the presence of the pathogenic type of Weil's disease can be ascertained by immersing guinea pigs in the suspected water when infection may occur through the moist skin.



Appelman (1934) after shaving and scarifying the abdomen of guinea pigs placed them in glass dishes containing water which had been infected with *L. pseudo icterogenes* and also *L. ictero-haemorrhagiae*. The animals only became infected with the latter organism. Van Theil (1937), however found it possible sometimes to infect guinea pigs with the *Leptospira pseudo icterogenes*. Water sodden skin facilitates the penetration of leptospirae.

In addition to water, in some experiments leptospirae have been found to survive in moist soil for as long as 3 months.

Hindle (1934) has isolated at least 8 water strain from different sources including 2 from London which all gave distinct serological reactions from the human strains.

**Other Methods of Infection**—It has been suggested that infection may occur in other ways than through the digestive tract and Inada believed such infections usual. There is no evidence that the disease is transmitted by insect bites or by direct transmission from man to man though the leptospira is sometimes present in human urine as well as in that of the rat.

Southwell (1938) relates that the bed bug (*Cimex lectularius*) was reported to have conveyed infection at an interval of 38 days after feeding on an infected animal but this observation has not been confirmed. Human infections with *L. canicola* have been demonstrated and in some instances apparently occurred from infected pet dogs. Uhlenhuth and Bloomberg (1937) have reported human infection from handling infected laboratory rats and in his laboratory 3 attendants acquired the infection almost certainly from rats. He cautions that attendants should wear rubber gloves and that care should be taken that no contamination of the conjunctiva occurs from infected urine of the rats. Goethals (1916) reported 2 cases of infection in laboratory workers from spraying infected material into their eyes.

## ETIOLOGY

The spirochaete causing infectious jaundice *Leptospira ictero-haemorrhagiae* is the type species of a genus described by Noguchi as having minute elementary spirals running throughout the body and failing to show either flagella or undulating membrane. The caudal portion of the spirochaete is remarkably flexible and when in motion the whole body seems drawn into a straight line except for the hook formation of one or both terminal portions. Propulsion seems to occur by the rotary motion of the hook and progresses in the direction of the straight end. If both ends become curved progression ceases. It is said to be insoluble in 10 per cent saponin thus differing from the other blood spirochaetes.

The organism varies considerably in size 6-14 (even 4-20) by 0.25 $\mu$ . The constituent spirals are closely placed together giving the appearance of small dots. As the organism is very slender its demonstration in the fresh state can usually be made only by means of the dark field illumination. It however may be stained by Giemsa's solution but in such preparations it is usually difficult to demonstrate the fine spirals.

It also may be well stained by one of the silver impregnation methods. Under the dark field the living organism is often minutely coiled, each coil appearing as a dot and the whole body suggesting a chain of refractile bodies. It is actively motile and often binds itself in the form of a hook, giving the appearance of the letter C when the hooks are on the same side or of the letter S. It is on account especially of the peculiar minute elementary spirals running throughout the body and resembling a coil of rope that Noguchi established a new genus for this species.

The organism is frequently found in the blood during the first 3 or 4 days of the disease. It is also present in the urine later and in a few instances it has been demonstrated in the cerebrospinal fluid and even in the sputum. Young guinea pigs are especially susceptible and following their infection death usually occurs with jaundice, albuminuria and haemorrhages. At necropsy spirochaetes are best demonstrated in films from the liver and in the kidneys. Under the discussion of epidemiology it has been noted that infection

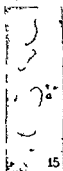


FIG 81

FIG 81—Four specimens of *L. pt sp m* (Aft r Nog h n J rnal f E p r m nt l M d c n )



FIG 82

FIG 82—A *L. pt sp a v w d* under the dark field microscope (Aft N gu h Jour al of E p e m ntal M d c n e)

with the organism is common among rats in various parts of the world and it may be considered a natural infection of rats as they do not seem to suffer from it.

**Cultivation**—*L. pt sp m* has been cultivated on Noguchi's leptospira media. It prefers a partial oxygen tension and usually grows in the narrow zone just below the surface. The optimum temperature for growth is from 25 to 30 C like many pathogenic organisms. Schuffner has recommended a culture media composed of tap water 1500 cc, Witte's peptone 0.15 gm, Ringer's solution 300 cc and Sovensen's solution pH 7. The final reaction of this peptone medium should be between pH 6.8 and pH 7.2. Three cubic centimeters of this medium is placed in a small tube and sterilized and for use 3 cc of rabbit serum is added. The tubes are then heated to 56 C for 30 minutes and incubated at 37 C overnight. The organism can also be grown on other forms of blood agar.

Morrow (1938) has succeeded in growing a human strain of *L. icterohaemorrhagiae* on the chorio-allantoic membrane of the fowl embryo. The spirochaetes were carried through 20 successive passages in developing eggs and after every 5 passage guinea pigs were injected. They became infected and died in 6-8 days with typical symptoms of Weil's disease.

The leptospirae are able to pass through the ordinary types of Berkefeld filters (N V W). Iida has obtained filtrates in which no spirochaetes were demonstrable but which were infective for guinea pigs. He believes therefore that there may be a viable granular form.

Although in leptospiral jaundice the organisms are often easily demonstrated in the blood of guinea pigs in many cases they are very difficult

or impossible to demonstrate in the blood of man either by direct examination or by inoculation. In sections of infected organs at autopsy they may be well demonstrated by Levaditi or Fontana's method.

**Serological Races**—In recent years a number of serological races of leptospira have been reported. Schuffner distinguishes 3 human strains in Europe: (1) the cosmopolitan *L. icterohaemorrhagiae* in rat and dog and the cause of classical Weil's disease; (2) *L. canicola* causing specific canine disease (occasionally transmitted to man); (3) *L. grippolyphosa* the infecting agent in the swamp fever of eastern Europe. He believes that the clinical (non-icteric) character, the serological and the epidemiological picture (appearing in well defined epidemics) are features which separate swamp fever from the other leptospiroses.

Korthoff infected a number of patients with swamp fever for therapeutic purposes. The disease was accompanied by high fever but jaundice never appeared. However Schuffner emphasizes that in dogs infected with *L. canicola* jaundice was not encountered and in his one human case infected with this strain there was also no jaundice. Nevertheless the strain pronounced *L. canicola* by serum reactions and isolated in California produced icterus in dogs as well as in one human case. Schuffner states that he has never found *L. canicola* in sewer rats and also that the white rat cannot be infected with *L. canicola*. Kaufmann (1938) also believes that by complement fixation, agglutination and flocculation tests *L. canicola* can be separated serologically from *L. icterohaemorrhagiae*.

In Japan besides *L. icterohaemorrhagiae*, *L. hebdomadis* and *L. autumnalis* have been encountered. *L. hebdomadis* is the cause of seven-day fever, a disease which occurs in parts of Japan and resembles a mild Weil's disease. The organism cannot be distinguished morphologically from that of Weil's disease but Ido, Ito and Wani were able to differentiate it by cross protection tests and by the Pfeiffer phenomenon. They found that the organism was carried by field mice and transmitted to man by contamination of the soil with the urine of the infected animals as well as by their bites. It is only feebly pathogenic for animals. Similar organisms have been found in cases of the disease known as Autumn fever in Japan. Koshina, Shiozawa and Kitayama have differentiated 2 species by their serological reactions. One was found to be identical with *L. hebdomadis*. The other *L. autumnalis* was more virulent and resembled *L. icterohaemorrhagiae* but did not correspond to either serologically. Abe (1934) reports the causative agent of Hasami in Japan to be *S. autumnalis*. He observed 9 cases in Nagasaki and says the strain is serologically distinct from *S. icterohaemorrhagiae* and *S. hebdomadis*. (See Chapter IX for further discussion of these infections.)

In the East Indies 8 strains have been described. Six of these were reported by Fletcher working in the Malay Peninsula and have been found to correspond with Dutch East Indian strains. Taylor and Goyle in the Andaman Islands found 2 more and Louwenaar and Wolff on the east coast of Sumatra have described 2 strains in dogs. Essenveld and Mochear (1938) found 2 strains in the field rats of Java, one identical with the human Sumatra strain while Vauzel (1937) reported in Indo-China, Netherlands India and the Malay Archipelago that the 3 Japanese types *L. icterohaemorrhagiae*, *autumnalis* and *hebdomadis* were present and in addition *L. grippolyphosa* and *L. canicola*. Fletcher (1934) separated his 6 strains from 26 patients by agglutination tests and Pfeiffer's reaction in guinea pigs. Some of them belonged to the *icterohaemorrhagiae* group which includes strains from dogs sent him by Okell who isolated them in England and also Noguchi's so-called yellow fever strains. Most of Fletcher's strains however were found to belong to the group represented by *L. pyrogenes* of Vervoort. Another large group was represented by the Akiyama strain (*L. hebdomadis*). There was no marked clinical difference between the cases in the different groups but the leptospira are serologically distinguished and an antiserum in treatment for *L. icterohaemorrhagiae* would have been of use in only a majority of the cases. Nevertheless there was a considerable overlapping of the antigens and a guinea pig which had recovered from infection with the strain of one group had a considerable immunity to infection with other races. Most of the strains were isolated

from black rats and some differed serologically from all the human strains. Only one was isolated from a dog.

The strain *L. febrilis* (Vervoot 1932) was isolated from an outbreak of pseudo dengue in Medan, Java. While Fletcher has regarded it as a distinct strain Schuffner believes it is only a strain of low virulence of *L. icterohaemorrhagiae*.

According to Fletcher and Brown there are a number of distinct serological races of *L. icterohaemorrhagiae*. They believe that the Andaman, Indian and Sumatran strains can be distinguished from those found in Europe and elsewhere.

There has been much difference of opinion about the advisability of regarding all these serological strains as distinct species, especially since there has sometimes been considerable overlapping in the serological reactions. Some investigators have attempted to distinguish species by the Rieckenberg or adhesion reaction, which is still more dangerous and of doubtful value for accurate differentiation. Hindle, Schuffner and Yorke (1934) incline to the view that it is inadvisable to regard many of the strains as distinct. Schuffner states the Indian strains should be classified in one variable species, though there may be some value in retaining the names. Sorgdrager and Schuffner (1938) have compared *L. canicola* and the typical Weil's strain with typical antisera. With the *L. canicola* antisera the reaction was positive in dilutions of 1:30,000 while the reaction with Weil antisera with this organism were never positive in dilutions of more than 1:3,000.

Van der Walle (1938) obtained a culture from the kidney of a dog. The serum of the animal produced lysis of this culture (*L. canicola*) in dilutions of 1:3,000 and of *L. icterohaemorrhagiae* in 1:10. However, Reiter (1938) has shown that *L. icterohaemorrhagiae*, *L. canicola* and *L. grippotyphosa* have a similar antigenic structure, though they may show many variations. *L. hebdomadis* however he regards as distinct.

### SYMPTOMATOLOGY

After an incubation period of from 6 to 12 days (rarely up to 19 days) the disease sets in abruptly with fever, rigors, headache, muscular pains and vomiting. The patient is often prostrated and has the appearance of being extremely ill, the face flushed and the conjunctivae injected. Some clinicians regard the intense injection of the eyes as the most striking early symptom. There is fever of an irregular type, usually running between 102° to 104° F. for the first 3 or 4 days, when it begins to fall by lysis, although occasionally by crisis about the fifth day. In severe cases the temperature may not decline until about the 10th day. Following a few days of moderate fever or normal temperature there is a tendency for a second rise toward the end of the second week, which continues for approximately another week, when a slow convalescence sets in in favorable cases. The secondary fever often shows rather marked oscillations.

Jaundice frequently appears about the second or third day with marked tenderness of the liver and sometimes slight enlargement of the spleen. The hue of the jaundice is yellowish rather than greenish and the skin

rarely itches. However, in some cases pruritis is present. Herpes is common and erythematous or papular rashes may occur. The urine is scanty and high colored, showing albumin and a large amount of urobilin. Early in the second week, urine of a low specific gravity is excreted in large amounts. It is usually bile stained. The pulse is rapid at first to become slow with the appearance of the jaundice. Its rate is then usually between 75 and 85 and the systolic blood pressure often is in the neighborhood of 120 millimeters of mercury. There is a tendency to sleeplessness and nocturnal delirium and in unfavorable cases the condition may resemble the typhoid state when skin rashes, petechiae and enlarged glands are common. Pains in the nape of the neck and calf muscles are common features. In some instances involvement of the central nervous system is apparent. There is stiffness of the neck and a positive Kernig's sign. Diagnosis of meningitis may be suggested. Murgatroyd (1939) has reported a chronic case of leptospiral meningitis in which leptospirae were recovered from the patient's cerebro spinal fluid six and eight months respectively after the onset of the disease, the spinal fluid having been successfully inoculated into guinea pigs. The patient's serum more than 2 years after his attack agglutinated the leptospira in high dilutions. Iritis and irido cyclitis have been reported as complications.

Haemorrhages starting as epistaxis, are commonly observed. In some outbreaks intestinal haemorrhage has been frequent. Haematuria is rarely observed. The red cells and the haemoglobin become reduced though marked anaemia is unusual. There is an increase in the polymorphonuclear leucocytes to about 15 000 to 20 000 in severe cases even more. The Van den Bergh reaction is directly positive. Azotemia is usually present during the first week and may become more marked later, the blood urea ranging from 50 to 397 mg. and death from uraemia may occur. The bilirubin content of the serum is often very high, and the direct Van den Bergh reaction is often obtained. During the second week antibodies, agglutinins and lysins appear. The leptospirae which are found in the blood in the early days of the infection soon disappear and after a week or 10 days may be found in the urine where they may persist for as long as 6 weeks.

#### SPECIAL SYMPTOMS

**Jaundice**—While jaundice is usually present in severe cases in the milder forms it may be absent. Inada (1917) found it present in the Japanese cases in about 60 per cent. Schüffner in Holland found in severe cases jaundice was absent in only about 13 per cent but in the milder ones it was absent in about 58 per cent. Fletcher found in the Malay States that the percentage of cases without jaundice might even be higher. In the 22 cases which have been reported in the United States jaundice was found to be a prominent feature in 21.

In the swamp fever of Russia, eastern Europe and parts of Germany jaundice has not been observed. Korthof who studied the disease experimentally and inoculated 11 individuals with the disease found 2 of them

refractory The remaining 9 developed the disease in from 5 to 9 days after inoculation The temperature reached 40 C (104 F) at times and sometimes higher However jaundice never appeared In cultures the invading leptospira resembled some of the species isolated from water but it was found to be pathogenic for guinea pigs

Prausnitz and Lubinski were not able to transmit to young rats the organism isolated from human cases of swamp fever While a number of investigators believe that this strain of leptospira from swamp fever is serologically different Kathe considers it identical with *L. icterohaemorrhagiae*

In seven day fever of Australia due to strains of *L. pomona* there is no jaundice but in Japan with that due to *L. hebdomadis* there may be a slight jaundice in some cases In the pseudo dengue observed in Deli Sumatra in which disease Vervoot isolated *L. febrilis* the jaundice is said to occur in some localities and not in others Haemoglobinuria was observed only once *L. febrilis* has been said to resemble serologically in some respects *L. icterohaemorrhagiae* and in others *L. hebdomadis*

**Conjunctival Injection**—The Dutch physicians have emphasized the occurrence and importance of the flushed conjunctivae According to Baerman and Smits the most constant and typical sign of a mild Weil's disease is the injection of the eyes They noted this appearance with very few exceptions in which there was a dull cloudy reddish leady luster without the yellowish background of an early jaundice They thought the mere raising of the upper lid and observing this condition was sufficient to make a diagnosis in the majority of cases The vessels of the bulbar conjunctiva and those of the sclera are all visibly distended beginning at the covering fold of the conjunctiva and falling off in intensity until the cornea is reached Sometimes only injection of the vessels under the eyelid is present The symptom may at times rapidly disappear but in other cases it may remain for a considerable time Kouwenaar (1930) in Sumatra and Kramer in Rotterdam have emphasized the importance of this conjunctival condition and Kramer observed it in about one half of the cases of Weil's disease without icterus The red eyes of these patients due to the flushing of the episcleral capillaries was said to render them conspicuous even at a distance and to be a valuable symptom even in typical cases of Weil's disease DeLangen believes this form of conjunctivitis is only seen in Weil's disease tropical typhus and pseudotyphoid (Kedani fever) Manson Bahr (1936) says that this injection of the conjunctivae presenting a distinct network of vessels on the cornea and sclerotic is almost pathognomonic He suggests that it is due to the primary invasion of the conjunctiva by the leptospirae However in connection with this symptom it should be recalled that Brown (1934) who has studied 4 outbreaks of epidemic jaundice comprising several hundred cases in all of which there was no evidence of spirochaetal infection found ocular congestion extremely common

**Clinical Stages.**—Inada in the study of the clinical course of the disease in Japan has recognized 3 stages febrile toxic and convalescent In the first or febrile stage lasting 6-7 days the disease usually begins with

a chill, high fever and prostration. More common symptoms are gastro-intestinal disturbances, abdominal pains, conjunctivitis, herpes labialis, severe muscular pains and signs of meningeal irritation. Examination of the blood shows a leucocytosis and numerous spirochaetes may be present. However antibodies are absent. Some degree of azotemia is usually observed. The urine contains albumin and casts but the organisms cannot be found in the urine. In the second or toxic stage jaundice appears in from 50 to 60 per cent of the cases. It usually begins on the 7th or 8th day and extends for 5 or 6 days. Many of the cases show haemorrhagic tendencies, and prostration, nervousness and cardiac symptoms may appear. Evidences of bleeding are frequent. The liver is usually enlarged, the spleen only rarely. Azotemia becomes more marked and there may be oliguria and even anuria. During this stage antibodies appear in the blood, but the spirochaetes disappear. The leptospirae, however, may be demonstrated in the urine. When death occurs it is usually in this stage.

The convalescent stage begins about the third week. A decrease in the icterus and azotemia takes place. The antibody production in the blood rises. The leptospirae are usually still demonstrable in the urine. A secondary rise in temperature occurs in from 28-40 per cent of the cases lasting from 5-14 days. During this period the blood urea may also be increased.

Davidson (1937) and Sladen (1939) have called attention to what they term a subclinical form of the disease in individuals exposed to infection. Thus Allston and Brown in a series of apparently healthy sewer workers found that 20 per cent of them showed positive serum agglutination reactions for *L. icterohaemorrhagiae*. Reese (1939) also points out that the disease does not always show itself in its well recognized and characteristic form. Meningitis may be the picture present. Murgatroyd also suggests that it may be wise to consider a possible diagnosis of Weil's disease in many cases of meningitis.

Davidson (1938) in the study of 130 cases of the disease among the fish workers in Aberdeen, in which positive serologic evidence of previous leptospiral infection was obtained, suggests that Weil's disease occurs in 3 grades: (1) latent or subclinical infection with or without insignificant symptoms; (2) mild infections with pyrexia and malaise but without jaundice; and (3) severe infections with jaundice. The first group can be recognized only by serologic tests. The second group may be suspected if the illness occurs in an individual working in an occupation which predisposes to Weil's disease. However serological tests are of course essential to confirm such suspicions. He believes the cases in Group 2 are often incorrectly diagnosed.

#### PATHOLOGY

Pathological changes are found chiefly in the kidneys, liver and skeletal muscles. Generalized jaundice is often present. Capillary and larger diffuse haemorrhages are frequently observed. They may be dermal

subserous mucosal or cerebral Haemorrhages also are usually present in the parenchyma of the liver kidneys and spleen The injury to the capillaries is presumed to be due to the toxic action of the spirochaetes It is sometimes so severe that it results in epistaxis haematemesis haemoptysis or purpura Frequently the calf muscles and sometimes other skeletal muscles show small haemorrhages which are often infiltrated by endothelial phagocytes In many instances the liver is slightly enlarged and appears jaundiced In some instances the liver cells are swollen in other instances granular degeneration may be present In cases when death has occurred later in the disease there may be more extreme cellular degeneration and often focal necroses In some instances fatty degeneration is present Usually however it is not more than moderate in degree and not of the character observed in yellow fever or in advanced acute yellow atrophy of the liver However Sefton (1938) who has reported a fatal case of Weil's disease from Brazil in which leptospira were demonstrated found lesions resembling those of acute yellow atrophy

Harris (1942) has compared the pathological observations in Weil's disease with those in yellow fever

The leptospirae can often be demonstrated in sections by Levaditi's method There is nothing characteristic in the appearance of the spleen which is usually only slightly swollen The substance is generally soft and diffuent The kidneys are often moderately swollen and jaundiced Sections of the kidneys usually reveal granular degeneration of the epithelium and necrosis in the convoluted tubules with infiltration of the interstitial tubules by lymphocytes and endothelial leucocytes Haemorrhages are frequent in the intertubular tissues Later in the disease the changes may resemble those of an interstitial nephritis and the leptospira may be found in considerable numbers In some instances submucous petechiae have been observed in the stomach and intestines

#### PROGNOSIS

The mortality has varied in different countries from 4 to 32 per cent in Europe to 48 per cent in certain Japanese outbreaks The virulence of the outbreak has varied in different countries and a varying susceptibility in individuals has been demonstrated In the *Maladie de la vase* or *Fievre de vase* the virulence of the infecting organism *L. grippotyphosa* is said to be low and that it is not virulent for rodents There is no jaundice in the human cases and attempts to infect 11 subjects failed in two instances demonstrating their increased resistance Schuffner (1934) found in Holland during 10 years 452 cases among which there were 46 deaths 10.2 per cent In his cases with jaundice from 1924-31 a mortality of 32 per cent occurred and in 1932-33 a mortality of 16 per cent He thought the lower mortality in the later years was due to the lessened virulence of the disease and in part to the effects of serum therapy the use of which had been considerably extended in Holland Schuffner believes no one dies of Weil's disease unless suffering from jaundice and that Weil's disease without jaundice is as harmless as any other leptospirosis which never shows jaundice in its course like Swamp fever or Nanukayama (seven-day fever) The mortality in the Andaman



Islands has been reported by Taylor and Goyle (1931) as 18.7 per cent and in the Isle of Syra (Greece) by Lorando (1932) as 12 per cent. In the sugar cane cutters of Queensland Cotter and Sawyers (1935) report a morbidity of 18 per cent and a mortality of about 4 per cent. On the other hand in 30 cases of seven day fever in Pomona Australia there was no mortality.

In the typhoidal, uraemic and meningeal forms the prognosis is usually very grave. It is especially grave when the cerebrospinal fluid is under pressure and contains an excess of albumin and leptospirae in large numbers. Nevertheless death does not always occur in such cases. Murgatroyd (1939) reported a recovery from a case of meningitis in which the leptospira was recovered from the cerebrospinal fluid months after the onset of the disease. Eschbach (1939) has also reported a case of meningeal encephalitis in a 12 year old child which was accompanied by adenitis and cutaneous eruption.

### DIAGNOSIS

Accurate diagnosis is made in the laboratory and depends upon (1) detecting leptospirae in fresh blood, (2) by culture from the blood (3) by

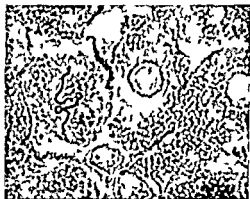


FIG. 83.—Leptospira terohaemorrhagiae in liver of a guinea pig inoculated with patient's blood. Leivad's stain  $\times 1000$ . (After Drs Havens, Bucher and Randall. Courtesy J. Am. Med. Assn.)

inoculation of the blood into guinea pigs or hamsters \* (4) detection of the organism in the urine and the inoculation of guinea pigs with it (5) serological examination.

In the first few days of the disease a search should be made with the dark field illumination for the leptospirae. Thick films may also be stained by Giemsa's solution for examination. The organisms are rarely numerous and are only present during the first days of illness so that they are frequently not detected. Better results have sometimes been obtained by centrifuging fresh citrated blood. Triple centrifugation has been recommended but Schuffner has emphasized the difficulty of precipitating the spirochaetes even at high speeds. He recommends centrifugation.

Randall, Morton and Larson (1944) have pointed out the value

gation of 10 minutes duration at 1500 revolutions when the plasma is separated from the precipitated red corpuscles he examines the thick layer. He recommends precipitation of the blood as well as examining the thick layer of the supernatant plasma. The plasma may be again centrifuged at 3500 revolutions per minute and examined for spirochaetes.

Taylor (1931) in some instances has been able to detect leptospirae in the blood as late as the 8th or 10th day. When not found in the blood 3 to 5 cc. of it or the centrifuged plasma should be inoculated into the peritoneal cavity of guinea pigs. If the infection is successful the leptospirae can frequently be detected in drops of fluid from the peritoneal cavity of the animal after several days usually being present by the third (Schüffner). The animal usually dies within 10 or 12 days with characteristic pathological lesions. The tissues are bile stained and the pleural and peritoneal surfaces are dotted with haemorrhages. Haemorrhages are also seen in the various organs especially the lungs. The liver is swollen and numerous leptospirae are present which are clearly visible in preparations made from it examined with the dark field.

After the first week of the disease leptospirae should be searched for in the urine. They are usually most prevalent from the 10th to 20th day of the disease. Centrifuged sediment of fresh urine should be examined directly and guinea pigs should also be inoculated with it. Inada reports that practically all cases of the disease showed organisms in the urine by the 20th day. However some observers have not been successful in demonstrating the leptospirae in the urine of their cases. Fletcher believes diagnosis is most easily made from cultivation in the incubator by direct inoculation of the blood into blood agar. Schüffner also recommends culture as a desirable method of diagnosis. He cultivated the organism from 51 cases of which 18 were without jaundice.

In the later stages of the disease after 6 or 7 days during convalescence and subsequently antibodies are present in the serum and diagnosis of the infection may be made by the agglutination test and also by Pfeiffer's phenomenon. Schüffner believes agglutination tests are best carried out with leptospira cultures preserved in 5 per cent formalin as they do not lyse so readily as the living organisms and he has found that the killed leptospirae agglutinate up to the highest dilutions compatible with the strength of the serum. If living leptospirae are used agglutination appears only in the lower dilutions as in the higher ones lysis sets in rendering agglutination impossible. However the formalized organisms are often rendered unsatisfactory for the test after some weeks since the leptospirae usually become matted together into felt like clots. Brown and Broom (1939) thought that cultures which have been quite recently formalized are not agglutinated to as high a titer as those formalized for 48 hours or more. They believe that there is no doubt that a florid serum will give a positive macroscopic agglutination test after one hour's incubation. They however think that the macroscopic method is not nearly as sensitive as the microscopic and that in certain serums of low titer such as those shortly after the 6th day of the disease the reaction may

not appear if the macroscopic test only is employed. Schuffner and others used the agglutination absorption test to differentiate various strains of leptospirae. The agglutination may be positive in a dilution of 1:100 after 6-8 days. According to Baermann and Smits the serum a few days later may have a titer of 1:500. The titer gradually rises and after about 50 days may be over 1:30,000. After this period it falls. They found that about 30 per cent of the sera of all patients might be negative after 200 days. However Postmus (1933) has reported that the agglutination may still be demonstrable in some sera after 8 years. Schuffner states that certain sera from cases in the Belgian Congo that gave a positive agglutination test for Weil's disease were negative with the mouse protection yellow fever test.

Packhamian (1941) has also employed the freshly prepared formalized antigen. He found the agglutination titer of 5 cases ranging from 1:300 to 1:1,000. In 8 cases the titers ranged from 1:3,000 to 1:10,000. The remaining 27 cases gave a titer of about 1:30,000 or higher. The agglutination reactions in these dilutions were prompt and completed within two hours. He concludes that the agglutination test when positive is of great value in the diagnosis of Weil's disease but that negative findings do not exclude the disease.

Elberton and Martorana (1942) in a serological study in New York City found 10 specimens of blood of 1:351 examined in which 10 gave a reaction of 1:320 or more. They regard a titer of 1:1,000 as indicative of a present or recent infection.

**Differential Diagnosis**—In some instances leptospiral jaundice may be confused with bilious remittent fever, syphilis of the liver, yellow fever, liver abscess, relapsing fever, and blackwater fever. Bilious remittent fever shows earlier jaundice, a more rapid pulse rate, and malarial parasites. In yellow fever there is a more marked rachialgia and earlier and more marked albuminuria. The marked leucocytosis of Weil's disease should be of value in differentiation. Faget's sign is not present in Weil's disease. The inoculation of guinea pigs with the blood or urine of the patient may give additional information. In yellow fever infection of guinea pigs at autopsy there is no jaundice or haemorrhage and leptospirae are absent.

The early jaundice and haemoglobinuria of blackwater fever should distinguish this disease. It seems probable that the reports of spirochaetes in blackwater fever came about from errors in diagnosis. The atropine test may help to identify typhoid fever from those cases of Weil's disease unaccompanied by jaundice.

**Immunity**—By the end of the first week of the disease antibodies become demonstrable and with their development there is a decrease in the number of organisms in the body and in their infectivity. Convalescent sera will protect guinea pigs from an otherwise fatal dose of the leptospira. The immune serum contains lysins which cause a breaking up of the organisms in the peritoneal cavity of the guinea pig (Ifeiffer's phenomenon) and also *in vitro* if fresh serum is used.

The *adhesion (or thrombocytobarin) phenomenon* has been suggested for differentiation of the various species of *Leptospira*. It depends upon the fact that the organisms are altered by their homologous antiserum in such a way that small particles in the mixture adhere to their surface. The nature of the particles is immaterial—platelets, leucocytes, living or dead bacteria, or inorganic substances. The serum must be fresh or reactivated by the addition of a small amount of complement. The phenomenon is a manifestation of a specific antigen-antibody reaction and depends upon some physicochemical change in the antigen. The technique is simple. Fresh undiluted antiserum is mixed with an equal quantity of a suspension of organisms and *E. coli*. After standing for 20 minutes a dark field preparation is examined. If the serum is homologous the colon bacilli will be crowded around the surface of the spirochaetes. Control preparations are necessary. This phenomenon has been obtained with trypanosomes as well as with spirochaetes. It is of interest but is not of great practical value.

For the diagnosis in wild rodents in and around Washington D. C. Larson (1943) has found that studies of sections of the kidney stained by Levaditi's method and examination of emulsions made from fresh kidney ex- yielded the greatest number of positive results.

## PROPHYLAXIS AND TREATMENT

**Prophylaxis** —As the infection appears to be transmitted through the medium of the urine and faeces sterilization of these discharges from those sick with the disease should be practiced. Extermination of the rat the host of the parasite is the important method of eradication of the disease. Scrupulous care to prevent food from being contaminated by the discharges of rats and mice also is of great importance in its prevention. In regions where the disease prevails and is endemic individuals should be cautious in regard to bathing and swimming and especially not to submerge the head in infected pools and sluggish rivers. Fish workers sewer workers and workers in damp mines should take care to prevent or protect abrasions of the skin which favor infections.

Since fish remnants especially attract rats and since the spirochaetes live in the slimy water care should be taken to remove all offal at the end of each day's work. The floors benches and tables of fish ware houses should be vigorously hosed with water and thereafter treated with a suitable disinfectant. Davidson has found a hyperchlorite solution in a dilution of 1:4000 has a lethal effect on leptospirae.

Laboratory workers must take precautions in handling infected animals and wear gloves.

In Japan prophylactic vaccinations with killed cultures of the organism have been tried. The results reported have been favorable but further investigations of their value are necessary. More recent work has been performed by Wani (1933). He has employed either an emulsion of the liver containing the leptospirae or pure blood cultures with the addition of phenol 0.5 per cent and the vaccine refrigerated for 7 days. He inoculated 10262 miners with this vaccine. Later there occurred among them a morbidity of 0.3 per cent while in the non vaccinated the morbidity was 1.12 per cent. He found that the serum of the vaccinated subjects after 15 months protected guinea pigs against a fatal dose of leptospirae. More recently Van Thiel (1938) has studied vaccination in Batavia by means of living avirulent cultures of leptospirae. He employed a strain that had been kept in culture for 8 years without passage through animals. Its behavior in guinea pigs indicated that it was avirulent or practically avirulent for these animals. He inoculated himself subcutaneously with 2 cubic centimeters of a culture of this strain of *Leptospira* and as a result passed through a very mild atypical attack of Weil's disease. He also inoculated 4 other persons all of whom also underwent atypical attacks. In 2 of them it was practically symptomless. In one the symptoms were mild but in another the symptoms were so severe that the volunteer had to stay in bed. The severe reaction was apparently due to the susceptibility of the individual. He points out that this case proves that the injection of avirulent strains of leptospirae are not entirely without danger and especially on account of the greater susceptibility of some individuals. He thinks that guinea pigs are evi-

not appear if the macroscopic test only is employed. Schuffner and others used the agglutination absorption test to differentiate various strains of leptospirae. The agglutination may be positive in a dilution of 1:100 after 6-8 days. According to Baermann and Smits the serum a few days later may have a titer of 1:500. The titer gradually rises and after about 50 days may be over 1:30,000. After this period, it falls. They found that about 30 per cent of the sera of all patients might be negative after 200 days. However, Postmus (1933) has reported that the agglutination may still be demonstrable in some sera after 8 years. Schuffner states that certain sera from cases in the Belgian Congo that gave a positive agglutination test for Weil's disease were negative with the mouse protection yellow fever test.

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dently much less susceptible to infection with *Leptospira* than human subjects

**Treatment**—This would appear to be largely symptomatic. Diet should be liquid and if vomiting is persistent it should be given as nutrient enemata. Mild cases require little treatment. In severe cases treatment may be indicated to counteract toxæmia and alleviate persistent vomiting and nephritic symptoms. The bowels should be kept moving freely and regularly. Intravenous injections of  $\frac{1}{2}$  to 1 litre of saline solution or Ringer's solution should be given. It is advisable to add 5 to 10 per cent of glucose to the saline solution. For severe cases serum treatment has been advocated. A polyvalent antiserum has been prepared in horses injected repeatedly with cultures of *L. icterohaemorrhagiae*. It is recommended that it be given intravenously at intervals of several hours for at least 3 or 4 days. Manson Bahr recommends 20 cc at least for each injection. For a man of 70 kg weight a dosage of 60 cc daily for from 3 to 5 days. DeLangen Schuffner and Manson Bahr emphasize that the serum should be given early in the disease if it is to be effective.

Tokuyama (1939) studied 9 cases in Hawaii 6 of which were treated by intravenous injections of immune serum. Four cases recovered and 2 died. However out of the 3 untreated cases 2 died. There was little evidence that the treatment had any effect although in 2 cases the injection was followed by general improvement.

Schuffner points out that if the serum is not given until jaundice appears its efficacy is very greatly reduced. Arsphenamine was tried in earlier years and found to have no effect on the infection. Hexamethylenamine has been recommended. Sodium tartro-bismuthate has been reported to give good results when injected subcutaneously into infected guinea pigs if it is given early in the disease. Heilman and Herrell (1944) infected 64 guinea pigs with *L. icterohaemorrhagiae*. 32 were treated with penicillin none of these died of the disease but 3 died from the toxic effects of penicillin. Of the 32 untreated guinea pigs 29 died of Weil's disease. From these experiments they suggest the possibility that penicillin may be effective in Weil's disease in man.

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## Chapter IX.

### RAT-BITE FEVER

(*Sodoku*)

#### DEFINITION

Rat bite fever is a relapsing type of fever following the bite of rats infected with *Spirillum minus* (*Spirillum morsus muris*) which bite served to introduce the virus. Following the healing of the wound the cicatrice shows inflammatory signs with lymphangitis and swelling of the tributary lymphatic glands. The onset of the disease is sudden with rigors and fever. The fever continues for several days then the temperature falls to normal and after an apyrexial period is followed by relapse. Numerous relapses may occur during the following weeks and months. In Japan the disease is known by the name of *sodoku* (from *so* 'rat, and *doku* poison).

#### GEOGRAPHICAL DISTRIBUTION

*Spirillum minus* is an organism as cosmopolitan as the rodents which produce it by the bite. Cases bacteriologically confirmed have been reported commonly in Japan and rarely in Great Britain, Holland, Germany, Italy, East Africa, the United States, the West Indies and South America, the Philippine Islands, Netherlands Indies, India and Australia. Shattuck and Theiler (1924) reported the first case in the United States in which the *Spirillum* was demonstrated. Since that time sporadic cases have been observed in the eastern, southern, central and western portions of the United States. Bayne Jones (1931) has collected 75 case reports from the literature of the United States from 1840-1930, which he considered genuine rat bite fever, in 5 of which the *Spirillum* was recovered. Since this time additional cases have been reported. It may be anticipated that cases of the disease, when carefully sought for, will be diagnosed in almost every country where rats prevail and live in close association with man. Ceccaldi (1940) has reported the first case from French Equatorial Africa at Brazzaville in which the organism was recovered.

#### ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—In a study of this disease Futaki and others discovered spiral organisms in the tissues of the bite area and the adjacent lymphatic glands (1916). They called the organism *Spirochaeta morsus muris*. The organisms were described as about 10 $\mu$  long including the terminal flagellae. In the blood of man and infected animals shorter and thicker spirochaetes were also found. While the organism was not found in man until 1915, Carter in 1887 in India discovered *Spirillum minus* in the

blood of the rat *Mus norvegicus*. Later it was found by Lingard in the blood of the bandicoot *Nesokia bandicota* and the guinea pig and rat were shown to be capable of infection. Subsequently it was found by Borrel (1903) and by Wenyon (1900) in the blood of healthy and cancerous mice. Puzs (1925) found in the examination of rats in Amsterdam that 3 of 260 were infected with a *Spirillum* which is pathogenic for the guinea pig causing an infection identical with sodoku. He however maintained that the *Spirillum* observed in the mouse while of identical morphology with that seen in the rat is not pathogenic. However Schockaert (1938) believes that they are the same species but that the organism under different conditions shows a variable virulence as could be demonstrated from experiments on animals.

*Spirillum minus* has not been found in the saliva but the transfer seems to occur by a break in the tissues containing the organism which is thus inoculated into the bite wound. It may possibly be excreted in the urine.

Manquelian (1940) has shown that in rats and mice the spirillum is attracted to the muscle fibers of the tongue. In silver preparations the organisms may be seen under the sarcolemma but they do not penetrate into the thickness of the muscle fiber. The spirilla escape from the muscle fiber to the surface of the tongue since especially at the top the muscles are covered only by a very thin epithelial layer which is easily broken.

**Morphology**—The organism is very variable in size. Most of the forms range from 2 to 5  $\mu$  by about 0.2  $\mu$  but much longer forms are occasionally seen. The coils vary in number depending upon the length of the organism and are uniformly spaced so that their crests are approximately 1  $\mu$  apart. The body is relatively rigid and one or more flagella are present at each pole. The flagella may be demonstrated by Burri's India ink method. The organism stains readily with the usual aniline dyes or by one of the Romanowsky blood stains and is Gram negative. Silver impregnation methods (especially the Tribondeau Fontana stain) are used to demonstrate them in the tissues. The motility observed in dark field preparations is unlike that of other *Leptospira* and resembles the rapid darting movements of the vibrios. The organism remains rigid and is apparently propelled by the flagella.

Since this type of locomotion is unlike that of other spirochaetes some bacteriologists consider that this organism should be placed in the genus *Spirillum* and refer to it as *Spirillum morsumuris* or *Spirillum minus*.



FIG. 84.—*Spirillum minus* in lung of mouse inoculated with blood from human rat bite fever. Silver impregnation.  $\times 1500$ . (From M. N. A. Aft. R. P. T. K. Fak. K. Tanguch and O. Um.)

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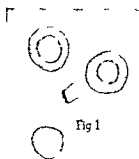


Fig 1

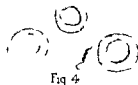


Fig 4

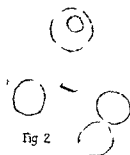


Fig 2

Fig 5

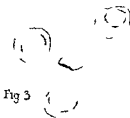


Fig 3

Fig 6

FIG 5.—1, 3 and 4. Sp. o. haet. of r. t. b. t. fever in gun a p. g. blo. d. F. nt. n. t. n. 5 and 6. Sp. h. t. f. t. b. t. f. v. n. g. u. e. a. p. g. bl. d. G. m. a. s. t. n. (Aft. Th. il. Dept. f. Trop. M. d. Ha. v. d. urt. y. Am. J. l. T. p. M. d.)

**Cultivation**—Futaki and several others have reported successful cultivation of the organism in special media. However Shattuck and Theiler and more recent investigators, have been unable to successfully cultivate it.

**Transmission**—*Spirillum minus* is found in the blood of infected mice, rats or guinea pigs during the first two weeks and then becomes distributed in the connective tissue, particularly around the lips, tongue and nose. They have not been found in the saliva and the transfer of infection by the bite appears to depend upon the existence of some break in the mucous membrane around the mouth. Mooser, working with experimental animals, has noted the frequent occurrence of infections in the eye and was able to find organisms in the conjunctival secretion. He suggests that this may be the source of the infection transmitted by the bite.

**Epidemiology**—In nature, a number of rodents serve as the normal reservoir of the virus.

The rat *Mus norvegicus* has been found infected in Japan in about 25 per cent while *Mus alexandrinus* was found infected in that country in about 3 per cent and the mole *Microtus montebellii* in the different provinces of Japan was found infected in from 12 to 54 per cent. In Bombay the black rat was infected in 2 per cent and the bandicoot *Nesokia bengalensis* in 11 per cent. In Calcutta about 2 per cent of a small number of rats revealed the *Spirillum*. At Caracas, South America, 10 per cent of the rats examined were found infected and in Amsterdam 3 rats out of 250 examined revealed the organism. Marcandier and Piro (1933) at Tuluon upon certain warships found 18 per cent of the rats infected.

The disease is much more prevalent in Japan than in other countries and it has been suggested that the construction of the Japanese houses gives greater opportunity for the occurrence of the bites of rats than elsewhere. Besides rats, bites from cats, weasles, ferrets and squirrels may cause a similar disease. Yamanato (1938) who has reported 3 cases of *sodoku* following bites of cats and Mollaret (1938), feel it is difficult to decide whether the cat is a true reservoir or whether it is an incidental transmitter from an infected rat which it has recently killed or eaten. The *Spirillum* can also be present in the blood of dogs showing no symptoms. Cazamian (1921) has reported a case and Ripley and van Sant (1934) 2 cases in which the infection was acquired from dogs. Ripley infected mice, guinea pigs and 2 dogs by the inoculation of the blood from a patient and spirilla were found in the blood of the mice. A patient suffering from dementia paralytica was inoculated with the infected mouse blood and developed a typical lesion and symptoms.

Arima (1934) has shown that it is possible to infect white rats by feeding them the organs of other infected animals. Levaditi (1934) found that *Spirillum minus* in white mice can be transmitted from the mother to the offspring either *in utero* or by the ingestion of infected milk. He believes that infection *per os* seems to be a certain method of propagating the disease among rodents and he found *S. minus* in the mammary glands of white mice. Also Russi (1938) has injected pregnant

ing the incubation period during which time the wound of the rat bite heals there is usually a rather sudden onset with headache nausea and marked weakness. The cicatrice now becomes inflamed and the surrounding tissues show oedema and at times vesicle formation. Even necrosis of the bite wound may occur. Leading from the inflamed areas there may be a line of tender lymphatics extending to a group of swollen lymphatic glands and in the course of this line induration of the muscles may be felt. There may be oedema of the hands and legs.

The onset is often characterized by chills and malaise. A rapid pulse and prostration are present during the pyrexial period. The fever rises rapidly to 101 F or 102 F and within 2 or 3 days has reached about 104 F and remains high for 2 or 3 more days. About this time

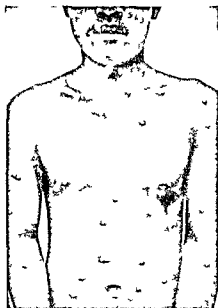


FIG 86—Purpuric eruption of rat bite fever (After Walsh)

it falls rapidly to normal attended with profuse sweating. The temperature remains normal for a few days during which time the local swelling and inflammation subside. An eruption of purplish spots or papules may accompany the fever appearing chiefly on the chest and arms. There may be urticarial lesions. Joint pains together with motor and sensory disturbances may be noted. Symptoms of nephritis may appear and the urine show albumin and casts.

Misoguchi differentiated four clinical types: (1) With general symptoms predominating; (2) With local ones mainly noted; (3) A type characterized by severe pains; (4) Cases where the neurological manifestations even paralyzes are prominent.

rats and guinea pigs subcutaneously and intraperitoneally with blood containing the *Spirillum*. Subsequent examination of the milk revealed the presence of the organism in smaller numbers than in the blood but they were still pathogenic.

On the other hand Das Gupta (1938) observed that 3 litters of mice infected with *Spirillum minus* failed to show any signs of infection and infection was not produced in mice by feeding them with urine containing this *Spirillum* or by feeding contaminated food. Schobl (1933) also found that fetuses of infected guinea pigs and mice showed no *Spirilla* indicating that there was no intrauterine transmission of the infection.

Arima (1933) has studied anew the role of fleas in the transmission of the disease in Manila. After the fleas were fed on infected rats the *Spirillum* remained alive for one hour after ingestion. They were still infective after this time when inoculated into guinea pigs. However after longer intervals infection was not produced. Attempts to transmit the disease by the bites of fleas gave uniformly negative results. There is also no evidence that other insects serve as vectors and no record of transmission from man to man by excreta or fomites.

Gupta (1933) has found *Spirillum minus* in the nasal smears of 4 cases of leprosy out of 3 000 examined in India. The nature of the *Spirillum* was proved by inoculation of the nasal scrapings into mice and guinea pigs and into human volunteers. The latter were inoculated with the blood of the infected mice and developed typical symptoms of rat bite fever.

#### PATHOLOGY

In guinea pigs or white rats inoculated with sodoku blood or ground up material from skin lesions, swelling of the lymphatic glands and of the spleen may result with sometimes the presence of spirilla in the blood. The liver may be congested and show a few organisms. White mice may appear healthy after inoculation or show conjunctivitis and occasional loss of hair. Spirilla are frequently demonstrated in the blood by direct examination. Guinea pigs usually succumb to most strains within 1 or 2 months after inoculation. They become emaciated and may show conjunctivitis, keratitis and loss of hair. Spirilla frequently cannot be demonstrated in the blood. Ripley and Van Sant however found spirilla in 3 cases in the discharges from the eyes of the animals. There have been few recorded human autopsies. Degenerative changes have been reported in the liver and kidneys. In some cases there has been increase in spinal fluid pressure and hyperemia of the cortex. Secondary infections with bacteria not infrequently complicate the disease.

#### SYMPTOMATOLOGY

The incubation period varies from 5-40 days or more the average being under 10 days. In two cases produced by inoculation it was 8 days. Greengard and Hess (1941) reported a fatal case in an infant of 11 months in which the incubation period was 12 days from the time of the bite of the rat. The infant died one month later. The specific organism was recovered by inoculation of mice with the blood of a patient. Follow

have collected from the literature 13 sporadic cases of rat bite fever and one of their own in which the organism was identical or closely related to the *Streptobacillus moniliformis* of Levaditi. Apparently the organism isolated by Schottmüller and others is also closely related to this strain.

In 1939 an epidemic known as Haverhill fever (*Erythema Arthritis cum Epidemicum*) was described in Massachusetts in which an organism similar to *Streptobacillus moniliformis* was isolated. Investigations by Strangeways, Tunnichiff and Lemierre have demonstrated that *Streptobacillus moniliformis* is one of the normal inhabitants of the nasopharynx of both laboratory and wild rats. In the cases of Haverhill fever reported by Place, Sutton and Willner there was no history of a rat bite. Arthritis was the most persistent symptom while true arthritis is absent in sodoku. Brown and Nunemaker (1942) have emphasized anew the importance of differentiating this infection from rat bite fever.

### DIAGNOSIS

*Laboratory diagnosis* of *S. minus* infections may be made by demonstration of the organism at the site of the bite or in material aspirated from a regional lymph gland. Occasionally the organism may be found in the blood in early cases. However it should be recalled that the spirillum usually has not been found either by microscopic or dark field examination of the peripheral blood or even in film preparations made from the swollen lymphatic glands. The organism has been more commonly demonstrated by animal inoculation. Guinea pigs are susceptible and usually succumb to the infection. In some instances it is difficult to detect the spirillum at autopsy. However care must be exercised to demonstrate that the animal employed for diagnosis is not already naturally infected before the inoculation of the suspected material is made. Mooser has reported latent infection in guinea pigs in which no clinical signs of illness developed. Das Gupta (1938) found in India 4 guinea pigs out of 4 harbored a natural infection with a spirillum morphologically identical with *S. minus*. Since both mice and guinea pigs may be spontaneously infected it is necessary to examine both the blood and the peritoneal fluid of any animals used for diagnostic examination.

Schobl has shown that it is possible to transmit the infection to the Philippine monkey (*Cynomolgus philippinensis*). However inoculation of monkeys with some strains is frequently without results. White mice and guinea pigs are more satisfactory for use in diagnosis. The organisms are often demonstrable in the blood of mice but in rats and mice infection of the blood may be transient and the animals rarely die from infection. In mice Ozeki reports that the infected animals can be recognized within 1 or 2 months after inoculation by the loss of hair on the belly and chest and the nasal line including the eyes and ears.

The successive waves of the disease with a syphilis like eruption and a negative Wassermann test in an individual bitten by a rat within 6 weeks of the onset of symptoms should suggest the diagnosis. However in some cases of sodoku a positive Wassermann reaction has been reported.



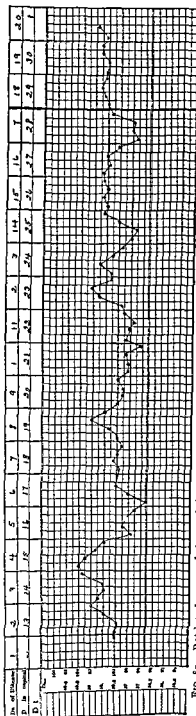


FIG 87—Rat bite fever in a baby aged three and one half months (Case of Dr George C Shattuck Courtesy Am Jour Trop Med)

After the critical fall of temperature there is usually an apyrexial period of several days during which time the local manifestations about wound and glands subside. The fever again comes on later to disappear and reappear. The successive paroxysms are usually of less severity and separated by increasing intervals. The fever is suggestive of the relapsing fevers. The pulse is rapid and weak. There may be as many as 12 of these febrile accessions and the course of the disease may extend over several months. There is an eosinophilia and during the febrile paroxysm a leucocytosis of about 15 000–20 000. The spirilla should be looked for in the blood during the early febrile periods. The dark field illumination is the best method for their demonstration. Should one fail to detect the organisms white mice or white rats may be inoculated with blood gland juice or emulsified tissue.

**Secondary Infections**—A number of investigators Schottmüller (1914) Blake (1916) Dick and Tunnichiff (1918) and Anderson and Spector (1932) have reported a streptothrix infection in rat bite fever. It is quite natural that the primary wound may become infected with other organisms than *Spirillum minus* and both cocci, bacilli and streptothrix have been encountered in lesions following the bite of rats. fatal infections of a septicæmic nature having sometimes followed. Levaditi (1926) reported an infection in a laboratory worker who came into contact with rats and recovered from his blood an organism which he named *Streptobacillus moniliformis*. Farrell Lordi and Vogel (1939)

## TREATMENT

One or 2 injections of salvarsan (arsphenamine) or some of its derivatives will frequently cure the disease. The usual doses of 0.3-0.6 gram are generally sufficient. Since recurrences are common in Japan when less than 3 injections are given a course of from 3 to 6 injections has seemed advisable. Otherwise the treatment is symptomatic.

Antimony preparations such as stovarsol and stibosan have also been used successfully by Schoekert and Schobl. Strychnine for the heart weakness and tonics during convalescence are recommended. Aspirin is often necessary to relieve the headache and joint pains. Radical wound excision of the local lesion is not recommended. As a prophylactic measure the same precaution should be taken to cauterize the wound as one would observe in rabies.

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Blum and Clement (1925) state that they found reports of 14 cases with positive and 22 with negative Wassermann reactions. They cite a case with a negative reaction before and a positive one after rat bite fever infection. Ripley and Van Sant found in 2 medical students in Chicago who developed the disease following the bite of a dog that the Wassermann reaction was weakly positive or negative during the duration of the infection but the Kahn test was positive. Das Gupta (1938) found that two experimentally infected human volunteers gave consistently negative Wassermann reactions but 4 guinea pigs infected from one of these cases all developed positive reactions. Savor and Lewthwaite have found that the sera of rabbits infected with uncontaminated strains of *Spirillum minus* (Rat Bite Fever) develop agglutinins for the O \ K strain of proteus in much higher titer than after infection with *Rickettsia tsutsugamushi*. From further experiments they concluded that there is an O \ K component in *Spirillum minus* unconnected with rickettsia in general and tsutsugamushi in particular and it is suggested that the common antigenic factor is a polysaccharide.

**Immunity**—The immunity conferred upon man and animals by an attack of the fever generally protects against the second attack. Ido Wani and Okuda demonstrated the presence of bacteriolytic antibodies in the serum of convalescents and the serum may agglutinate the organism in low dilutions. Das Gupta experimentally infected a human volunteer who had 3 bouts of fever and after treatment with arsphenamine recovered. He was reinfectd with the *Spirillum*, first 2 months and again 8 months later but proved refractory to further infection.

### PROGNOSIS

Miyake has reported in Japan in untreated cases a mortality of about 10.5 per cent. However since the introduction of arsphenamine therapy by Hata the deaths have been rare. Most of the fatal cases have occurred during the first severe febrile attacks although some have resulted later from nephritis or other complications. Bacterial infections of streptococci, streptococci or staphylococci frequently occur and may complicate the prognosis.

Solomon and others have employed experimentally induced rat bite fever as one of the diseases for the treatment of general paralysis. The disease thus produced may be severe and have undesirable features. Its value in the treatment of general paralysis has not yet been definitely established.

### PROPHYLAXIS

Effective prophylaxis depends upon rat destruction and the prevention of contact with rats especially in darkened areas where rat bites are more likely to occur.

In Manila it has been found that the distribution of rat bite fever and plague are coextensive and that measures taken against one disease are also effective against the other.

**Symptomatology**—In the previous edition of this book seven day fever was classed with the dengue like fevers because it at times showed a typical saddle back fever course. The onset is abrupt and the period of fever is attended by prostration muscle pains headache and anorexia. The lymphatic glands are enlarged in almost all cases and occasionally a measles like eruption has been noted on the forearms. Conjunctival congestion is common and there may be a very slight jaundice although some observers have failed to find this more distinctive feature of Weil's disease. Albuminuria is common.

It is often confused with dengue in proof of which seven day fever has been described as showing a leukopenia with polymorphonuclear decrease as for dengue while the Japanese observers note a leucocytosis. Some case reports show a relatively slow pulse for temperature as in dengue. Future studies may show that some of the cases belong to the dengue group and others represent very mild attacks of Weil's disease.

The agglutination test may be of assistance in distinction. The blood of convalescents can often be demonstrated to contain specific immune bodies and when such blood is inoculated into the abdominal cavity of a guinea pig with the culture of the leptospira a positive Pfeiffer's reaction may be obtained. Fletcher has isolated leptospira in the Malay states from a variety of febrile cases some resembling dengue. He has separated these organisms into 6 groups or strains but they are more or less related serologically. The Dutch investigators in Sumatra have also found leptospirae in fevers of from 1-5 days duration with no jaundice as well as in more severe cases with jaundice and finally in 2 cases resembling blackwater fever.

**Treatment.**—There does not seem to be any satisfactory specific treatment and one should treat symptoms as they arise.

#### PSEUDO DENGUE OF JAVA

##### (*Leptospirosis Febrilis*)

Vervoort (1923) studied in Java an unclassified fever in which he found a spirochaete which he named *Leptospira pyrogenes*. The organism was found in the blood from the first to the sixth day of the disease. It was demonstrated more often by cultures than by direct examination of the blood. It was also isolated from the urine later in the disease. The organism was pathogenic for guinea pigs and resembled serologically in some respects *L. icterohaemorrhagiae* and in others *L. hebdomadis*. The disease provoked by it was reported later as endemic in the plantations of Deli Java and was characterized by an acute onset and an attack of fever of 6 to 7 days duration resembling dengue. Icterus was variable being present in the cases in some plantations and absent in those from others. Severe headaches and pains in the muscles especially in the legs were noted. The fever was irregular and lasted from 2 to 15 days. In 10 per cent of the cases there was an erythematous or papular eruption. The Van den Bergh reaction was directly positive. In the cases with

## Chapter X

### OTHER FORMS OF LEPTOSPIROSIS

Other related forms of Leptospirosis have been described especially under the names of Seven day fever, Autumn fever Pseudo dengue Akiyama disease, and Marsh or Swamp fever

#### SEVEN DAY FEVER

This may be defined as a dengue like (or saddle back) fever of about seven days duration caused by a spirochaete, *Leptospira hebdomadis*. It has been studied chiefly in India and Japan, but probably has a wider distribution. The disease is known in Japan especially under the names of Nanukayami and Sakushyu fever. It is also sometimes termed autumn fever.

**Etiology**—There is evidently quite a close relation between *L. hebdomadis* and *L. icterohaemorrhagiae* but Ido and his colleagues claimed differentiation by serological means. Tagawa (1935) reported his studies as inconclusive. More recently Schuffner (1934) and Reiter (1938) have likewise reported differences in agglutination between *L. hebdomadis* and *L. icterohaemorrhagiae*. Schuffner also emphasized as a distinction that the former causes epizootics in voles. Fletcher (1934) in the Malay States has in addition isolated a strain corresponding to *L. hebdomadis*.

Mochtar and de Redde (1941) have reported Manukayami disease from Java: the sera of a number of the cases agglutinated (*L. hebdomadis*) in a dilution of 1:25,000. From 3 of 4 cases the leptospira was cultivated from the blood and urine in Noguchi's medium.

The parasite is found with difficulty in the blood of patients from the second to the fourth day of the disease. It is cultivable in the same manner as the spirochaete of Weil's disease. It is also found in the urine. When the blood of patients is injected into young guinea pigs a febrile condition is produced which is often fatal and the spirochaetes are found in their blood and organs. These animals show jaundice in only about 17 per cent while the findings with *L. icterohaemorrhagiae* approximate 100 per cent.

**Epidemiology**—The disease affects those who work in the fields of certain Japanese districts and the reservoir of the virus is the short eared field vole or field mouse *Microtus montebelloni*. The spirochaete has been found in the kidneys and urine of about 3 per cent of these (wild) field mice. The disease is found especially among workers in certain forests where the rodent prevails. It has been suggested that transmission may occur through the bite of this rodent but it would seem also probable that infection is connected with the spirochaetes given off in the urine. The spirochaetes should be searched for in the urine of man at the end of the febrile period and may continue to be eliminated for 4 or 5 weeks. There have been no fatal cases reported and no distinctive pathological anatomy

*Leptospira grippotyphosa aquatilis* Swamp fever and the question of the specificity of this strain has already been discussed in Chapter VIII

Schuffner (1918) found a leptospira resembling morphologically *L. icterohaemorrhagiae* in a patient who died of blackwater fever. The spirochaete was abundant in the blood, liver, lungs and kidneys. The blood showed heavy infection, but when inoculated into guinea pigs gave negative results. In 1934 Schuffner noted again that he had observed leptospirae which he did not succeed in cultivating in 2 cases of idiopathic blackwater fever not caused by malaria in Deli, Sumatra. No additional reports of this nature are known.

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jaundice the urine showed urobilinuria albuminuria, and true nephritis was sometimes observed. Haemoglobinuria was noticed in one case. The disease was in general benign death being observed in 2 or 3 per cent of the cases. It was distinguished from dengue by the presence of the albuminuria the absence of leukopenia and the ease with which one could demonstrate the spirochaetes. Kouwenar, in his thesis published in Amsterdam in 1924, differentiated between (a) this dengue like form of fever *Spirochaetosis febrisimplex* and (b) *Spirochaetosis febrilis cum ictero*.

Fletcher (1934) states that most of the strains of leptospira he studied in the Malay States belong to the group represented by the strain *L. pyrogenes* of Vervoort. However, Schuffner formerly suggested it represented a strain of *L. icterohaemorrhagiae* and the disease a mild type of Weil's disease.

### AKIYAMI DISEASE

#### (Autumn Fever)

This fever which was described particularly from the Province of Schizuoka in Japan was reported to be due to two species of leptospira A and B, morphologically identical with *L. hebdomadis*. More recently serological studies have shown that the leptospira of type B is identical with *L. hebdomadis* while the leptospira of type A (*Leptospira autumnalis*) according to Koshina and Schiozowa is different and very virulent for guinea pigs. Abe (1934) also found the causative agent of the disease known as Hasami in Japan to correspond to *S. autumnalis*. He reported 9 cases of infection in Nagasaki and found the organism to be serologically distinct from *S. icterohaemorrhagiae* and *S. hebdomadis*. Some writers regard Akiyami disease as identical with Seven day fever (Nanukayami).

### MARSH FEVER

#### (*Maladie de la Vase and Fievre de Vase schlammfieber*)

This disease has been observed on various occasions since 1880 in Silesia Saxony and Bavaria and Russia (Moscow), and is an icteric disease which has been observed especially in epidemics or outbreaks at times of inundation among people who are obliged to work or live in swampy regions. Numerous investigators have emphasized the importance of such a condition in the etiology of the disease as implied by the names swamp or marsh fever. The incubation period of the infection has been reported as from 5 to 9 days. The fever in some instances has reached 104°F and even higher. However no fatal cases have been reported. Jaundice has not been observed. The leptospira has been isolated from the blood during the incubation period of the disease and during the first two days of it. It is pathogenic for guinea pigs but apparently the organism isolated from the human cases is not pathogenic for small rodents. Tarassoff (1935) who found the organism named it

It is interesting to read the views of Sydenham as to the origin of the great epidemic of syphilis at the end of the fifteenth century. But to me it rather seems to have taken rise from some nation of the blacks upon the borders of Guinea for I have been informed by men of great veracity who have lived in the Caribbee Islands that the slaves which are newly brought from Guinea even before they land and likewise those that live there are afflicted with the disease (innocently). Also that it seizes the whole families—men, women and children.

And as far as I can learn this disease which so frequently attacks these miserable people does not at all differ from that we call the venereal disease with respect to symptoms—pains, ulcers etc. allowing for diversity of climate. But it goes under a different name for they call it the yaws. Nor does their method of treatment differ from ours for they carry it off with a salivation raised by quicksilver.

It seems to me that the disease was brought into Europe by Spaniards who first contracted it from negroes they had purchased in Africa in parts of which the disease may be endemic for the barbarous practice of exchanging natives for European merchandise prevails in many places on the border of Guinea. This contagious distemper spreading rapidly would have made the world a hospital and destroyed mankind. But like vegetables transplanted from its native place to a foreign climate it flourishes less in Europe. It languishes daily and its symptoms grow milder.

Much has been written of the possible identity in earlier years of yaws and such diseases as button scurvy of Ireland, sibbens of Scotland and radesyge of Norway and Sweden. Button scurvy was endemic particularly in the rural districts of the southern counties of Ireland in the eighteenth and early part of the nineteenth centuries. The descriptions of it suggest the possibility of its having resembled yaws. The disease was described as occurring only among the country people and its transmission ascribed to a transfer of the virus by fomites. The lesions were reported as reaching the size of a pea or a nut following their development from itching spots. They were covered with a dry crust which reformed if removed. The favorite seats of the eruption were the palms of the hands and the inner sides of the thighs and the arms. They were rarely present on the hairy scalp and when on the scrotum or perineum could easily be mistaken for condylomata. After lasting for some time they shrivelled, the scabs fell off and only a red spot remained.

In connection with sibbens which prevailed in Scotland in the seventeenth and eighteenth centuries and with radesyge a disease reported from Norway and Sweden in the eighteenth and early portion of the nineteenth centuries we apparently have counts of a severe form of syphilis.

**Geographical Distribution**—Yaws is essentially a disease of the inhabitants of warm countries. The disease is delimited by the Tropics of Cancer and Capricorn. Today it is common in the West Indies, tropical America and throughout equatorial Africa. It is less common in Tripoli, Algiers and the Sudan. In the Far East it is prevalent in the Malay States, Ceylon, Siam, Netherlands India, Burma, French Indo-China, the Philippines and the East Indian Islands and other Pacific Islands particularly Samoa. It is also present in North Australia. In most parts of India and China it is rare. In parts of the West Indies and Central Africa and in Fiji and Samoa the great majority of the natives suffer an attack of the disease in childhood. Recently it has been very prevalent in Kenya, Tanganyika and Uganda and is said to be spreading rapidly. In Haiti it has been estimated that 80 per cent of the rural population are infected and during a number of years mass treatment was given annually to some 400,000 cases. Manson Bahr (1940) states that yaws has disappeared to a great extent in recent years from Guiana, Barbados and even Ceylon where it was previously rife.



## Chapter XI

# YAWS OR FRAMBOESIA

### DEFINITION AND SYNONYMS

**Definition** —Yaws, or framboesia, is an infectious, contagious tropical disease, characterized by an initial cutaneous papillary lesion followed by a multiple, papular granulomatous eruption upon the skin and in some instances by late destructive lesions especially of the skin and bones. It is caused by *Treponema pertenue* found in the serous discharges of the yaws lesions and the lymphatic glands. It readily yields to treatment with salvarsan.

**Synonyms** —In the English speaking parts of the tropical world the designation yaws is the usually accepted name while in the French possessions the word "pian" is equally common. In the literature of the disease the term frequently employed is framboesia, a name applied by Sauvage because of the supposed resemblance of the fungous lesion to a raspberry. On account of the ambiguity of the designation framboesia Charlous proposed the name 'polypapilloma tropicum'. The colloquial names for the disease in various parts of the tropical world are Angola, 'momba' Ceylon, parangi, Malay Federated States, 'puru' Java, patek, Brazil, bubas, Fiji, coco, Samoa, lupani or 'tono', New Caledonia, tonga, Gold Coast, 'dube'.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** —Hillary tells us that the disease described in the thirteenth chapter of Leviticus was probably yaws but admits that the description which Moses has given us is so short and indefinite as to make this a matter of doubt. Some authorities think that a disease described by the Arabian physicians of the 10th century was yaws but the first description of what was undoubtedly yaws was that of Oviedo, who in the 16th century described such an affection as existing in the West Indies. Bontius later on noted its existence in the East Indies as well as in the West Indies. According to Labat it was especially prevalent among the Caribs.

It is known that yaws often occurred in epidemic form on board the slave ships and it is thought that this disease may have been an African importation into the New World. It became extremely common in the West Indian slaves brought from Africa. Brickell (1737) noted that in North Carolina yaws was very prevalent in the African slaves of that colony.

**Etiology**—The treponema of yaws *Treponema pertenue* discovered by Castellani in 1905 is morphologically indistinguishable from the *Treponema pallidum* of syphilis.

It is characterized by the same sharp cut corkscrew spirals as the syphilitic spirochaete discovered by Schaudinn earlier in the same year. *Treponema pertenue* is found in the epidermis of the yaws granuloma and has been demonstrated in lymphatic glands and spleen and in some instances in the bone marrow. Although it has not been demonstrated in the blood through microscopical examination it must exist there as monkeys infected with the blood of yaws patients may develop the lesions of yaws with the spirochaetes present.

**Cultivation**—The organism found in yaws is very difficult to cultivate. Its cultivation was formerly reported in Noguchi's ascitic fluid media containing a piece of fresh animal tissue the media being covered with a layer of sterile paraffine. However a number of investigators have since failed to cultivate it and such spirochaetes as have been obtained in cultures were avirulent for animals. It is also doubtful if the organism of syphilis *T. pallidum* has been successfully cultivated. Zinsser and Bayne Jones (1939) hold this view.

**Animal Inoculation**—Monkeys and rabbits can be infected by inoculation with discharges from yaws lesions. In rabbits most observers have described dry lesions following skin inoculation with yaws whereas with syphilis they resemble chancres. Turner and others reported that the epididymo orchitis in rabbits following inoculation with yaws was much less marked than with syphilis.

**Monkeys**—The work of Schöbl (1928) has been so extensive and so painstaking that it would seem well to outline briefly a few points in his study of yaws in the monkey. He inoculated *Cy. molg. philippinensis* with emulsified material from yaws lesions showing spirochaetes by the dark field examination. The injection was made intradermally with a small hypodermic syringe. Most of the inoculations were made into the skin of the nose, eyebrows and scrotum. The latter sites gave the best takes—that of the eyebrows giving a rather dry type of lesion and that of the scrotum a large lesion with abundant supply of yaws material. Of 16 eyebrow inoculations 14 were successful and of 7 scrotal ones all took. In from 3 to 5 weeks after inoculation on the eyebrow there appeared either a single papule, multiple acuminate papules or a flat indurated papule. These primary lesions extend as raised indurated oval plaques with unbroken surface until they may measure one half inch or more. Later on fissures or erosions appear on the surface and these soon become covered with a rather soft brittle crust of amber colour—in larger lesions the color of the crust may be darker from blood admixture. When the scabs are removed an ooze of slightly bleeding granulation tissue is uncovered. This yaws lesion corresponds to the typical yew in man. Later there is a spreading toward the periphery and an apparent healing in the center (ringworm form). As these lesions spread they become drier and resemble circinate psoriasis more than ringworm. When the inoculation was made on the scrotum there followed a moist spreading lesion surrounded by an area of intact skin infiltrated by a rather hard oedema. In some places the margins were rather undermined. An elevated granulomatous lesion formed, covered with brownish yellow scabs and surrounded by a slightly elevated reddish zone. The lesions spread with only slight healing in the center and may involve the entire half of the scrotum and base of the penis before regressing. In the monkeys inoculated for the first time the disease runs its course without developing the secondary or metastatic lesions (one exception) as seen in man.

## ETIOLOGY AND EPIDEMIOLOGY

Hutchinson, who especially studied syphilis insisted upon the syphilitic nature of yaws, and more recently a number of observers have concurred in that opinion. Perhaps the most conservative view in this respect is that we have in yaws a modified virus of syphilis—a less virulent one producing a disease which has been modified through many years of successive passage of the virus through the epidermis in black skinned races by the habits of life of these people and by the climate and hygienic

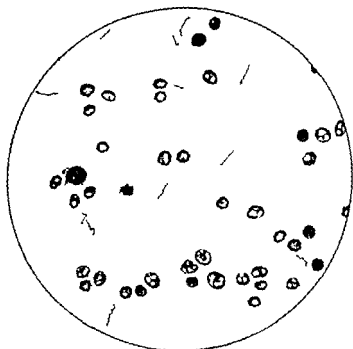


FIG. 88.—Camera lucida drawing *Spirochaeta perennis*. Specimen stained in Giemsa's solution. Magnification: Zeiss Compensating Ocular 6, Apochromatic Objective  $M_2$ , numerical aperture 1.40. (From Medical Report of Rice Harvard Amazon Expedition, 1926.)

conditions under which they live. There is much evidence in support of this view. Chandler (1940) points out that whether or not yaws was originally evolved from syphilis or vice versa, or whether under suitable conditions alterations can still occur, are largely academic questions. However, practically at least in its early stages, yaws is a recognizably distinct disease and one which the physician in the tropics should be familiar with. The relationship of yaws to syphilis has been most carefully studied for many years by Admiral C. S. Butler and many of his views, especially in recent years, have been generally concurred in.

Levaditi and Nattan Larnier reported success in inoculating yaws monkeys with syphilis but failure to inoculate syphilis infected monkeys with yaws virus thus suggesting yaws may be a mild form of syphilis.

Other experiments in rabbits by a number of observers have also indicated an immunity to yaws in animals first infected with syphilis and a partial immunity to syphilis in those infected with yaws. From Samoa, Fiji, Guam and other parts of the world where yaws is almost universal in the natives we have reports from careful observers that these natives are immune to syphilis although exposed to syphilis from intercourse with syphilitics of other races.

Jahnel and Lanke made attempts to inoculate general paretics with yaws virus obtained from a strain carried by passage through rabbits (a Nichols strain) but without a single take. Later on they inoculated 8 cases of general paresis with the strain of yaws spirochaete obtained from Pearce and Brown with unsuccessful results. As a control a case of multiple sclerosis responded to inoculation.

Van der Schaar (1933) also inoculated yaws spirochaetes into 40 individuals with paralytic demyelitis without result except in 1 patient who however had a negative Wassermann reaction.

Ashburn and Craig succeeded in inoculating monkeys with yaws but were unsuccessful in their attempts to inoculate *Cynomolgus philippinensis* with syphilis. The rabbit responds to intratesticular inoculation with yaws virus as well as with that of syphilis but Nichols thought that with syphilis the nodules were larger and the degenerative changes more active.

Differences in virulence of the syphilis and yaws viruses as well as of individual strains of them are suggested by some of these inoculation experiments in man and animals. However the susceptibility of the host may in some instances also have influenced the results. It has also been shown by some studies in experimental yaws inoculation that the treponema does not survive in the lymphatic system after the lesions have healed as it does in syphilis. Turner and Chancery (1934) have compared the experimental yaws infection in rabbits with syphilis in these animals attention being focused on the incubation period, initial lesions after intratesticular and intracutaneous inoculation, metastatic lesions and seasonal variation. The results showed that no differences were noted in the rabbits inoculated with 8 different strains of yaws virus. The disease picture produced by the yaws virus presented striking and for the most part constant differences from that produced by the Hitt strain of syphilis. This strain of syphilis gave rise in rabbits to a disease which was similar in every way to that produced by several strains of syphilitic virus isolated in the temperate zone. These results lend support to the view that yaws and syphilis are not identical.

Takahashi (1937) injected *S. pallidum* and *S. pertenuis* into the testes and skin of rabbits. He found the essential point of difference was the distribution of the organism widely into the tissues with mucoid degeneration andessel walls in the case of *S. pallidum* but congregated in heaps without mucoid degeneration in the case of *S. pertenuis*. The syphilitic animals showed spirochaetes and necrosis in the epidermis and corium of the skin but the yaws lesions were usually confined to the epidermis. *S. pertenuis* was seen only seldom in the corium and not at all in the subcutaneous tissues. It was abundant in the epidermis and the peripheral cell layer of the outer hair sheaths. The distribution of *pertenue* in the rabbit skin tissues is in accordance with that found by Schüffner, Seibert and Hallenberger and others in the skin of human beings suffering from yaws. Takahashi also found changes in the aorta in 68 rabbits infected with syphilis and none in 13 infected with yaws.

Ferris and Turner (1938) in their studies in Jamaica with 3 strains isolated from yaws and from syphilis found pronounced and consistent differences in the evolution of the lesions caused by *T. pertenue* and *T. pallidum* when inoculated into the skin of rabbits. The initial lesion was produced more rapidly with *T. pallidum* than with *T. pertenue*. *T. pallidum* produced more well developed lesions which did not subside as did those produced by *T. pertenue*. However in lesions of comparable size the histological changes are qualitatively the same in yaws and syphilis consisting predominantly in lymphocytic infiltration about hair follicles and small blood vessels. The

By superinfection or again inoculating the monkeys presenting existing or healed primary yaws lesions Schöbl was able to produce generalized yaws in the monkeys. He notes that the secondary lesions are more characteristic than the primary one. The secondary yaw may resemble the primary one but more commonly it is smaller, elevated, sharply outlined and oval. There is a dark crust which abruptly vanishes into the normal skin. Upon removing the crust we have an oozing granulomatous papillomatous formation which in appearance resembles a raspberry. These inoculations of animals, in some of which the primary yaws had spontaneously regressed, show that reinoculability of animals cannot be used as a criterion of therapeutic sterilization. In some of the superinfected monkeys the yaw spread from the eyebrow down the nose and cheeks reaching the mucous membrane of the nose. Even with healing of the skin lesions the process may continue in the nasal mucosa which started however from continuity with the skin lesion. Schöbl thinks that the spread of the gangosa ulceration can occur only in man or an animal in an allergic state—the monkeys with similar extensions to the nasal mucosa but not superinfected, never showing the destructive mutilating processes of gangosa.

Other late yaws manifestations such as lupus like or other ulcerative lesions and keratoderma of the plantar surfaces are similar in the monkey to those seen in man.

**Human Inoculation**—The fact that by means of the secretion from the yaws lesions the disease could be transferred to another person was well known to the slaves of the West Indies and furthermore they practiced autoinoculation in those children not showing a generalized eruption. Schöbl explains the occurrence of the disease in adults by the fact that they failed to have generalized yaws in childhood and thus did not acquire sufficient immunity to prevent infection, and it may be that this fact was known to the African native centuries ago.

Paulet in 1848 successfully inoculated 14 negroes, the eruption in 10 cases appearing at the site of inoculation. Charlois performed similar experiments and in addition he inoculated a yaws patient with syphilitic virus and obtained a chancre at the site of inoculation. This experiment should not perhaps be accorded the importance that was heretofore attached to it, as Schöbl has shown that immunity even to yaws itself is not acquired while the secondary dissemination continues. It has been held that immunity to syphilis is acquired much earlier than is that to yaws. Still Chesney and Kemp have shown that with syphilis in rabbits the infection must obtain for over three months to bring about immunity; if the animal is cured with arsphenamine before this time reinoculation is successful. There would seem to be a true immunity apart from latent infection.

**Relationship to Syphilitic Virus**—Inoculation experiments in animals (monkeys and rabbits) have shown that while the animals which have been inoculated primarily with yaws virus may sometimes be successfully reinoculated with the virus of syphilis, on the contrary animals inoculated with the syphilitic virus are often immune subsequently to yaws infection.

However recently Schöbl has shown that monkeys who possess a high degree of immunity to yaws produced by repeated inoculations of yaws virus are also subsequently immune to cutaneous inoculation of a strain of syphilis virus. A complete immunity to yaws apparently does not develop in the monkey until about 7 months after its primary infection.

of Assam yaws only gave condyloma like lesions about genital perineal and axillary regions but when these people went down to the hotter plains they developed florid yaws. Also Sellards Lopez Rizal and Hasselmann (1931) found that while yaws is widespread in the mountains of Northern Luzon (Philippine Islands) at an altitude of approximately 800 to 1200 meters the yaws cases observed in the mountains showed a striking peculiarity in that the cutaneous lesions in 90 per cent of the patients were limited to mucocutaneous junctures of the mouth nose anus and genitalia.

In some countries it is largely limited to rural districts in which syphilis is rare whereas in the towns yaws is uncommon and syphilis venereally acquired is prevalent.

Infection is usually acquired innocently in childhood. The spirochaetes may enter the body through some cut or abrasion of the skin either by direct contact with discharges from the lesions or indirectly particularly through the agency of flies. The greater the attention to personal hygiene the less probable is the spread of yaws which is one reason that Europeans are rarely infected although the disease may be prevalent in the native population. In countries where it is prevalent it is chiefly a disease of children the adults possessing immunity as the result of attacks in childhood. White children often are additionally protected when they are kept clothed and clean.

The spirochaetes are not able to penetrate the uninjured and unbroken skin. Unabraded skin surfaces offer a barrier to infection but in the tropics native children are frequently naked and suffer from numerous abrasions or skin ulcerations which provide a suitable point of entrance for the virus whether introduced by direct contact with a lesion on another child or by indirect transmission through contaminated material.

**Transmission**—Yaws is a readily communicable disease and man and monkeys may be successfully infected with material from one of the lesions of the skin with the production of typical *granulomatous papillomata* in the inoculated subject. Auto inoculation also may occur during the early course of the disease in man and the infection spread to other parts of the skin and mucous membranes not already invaded. Lesions may also be produced in the testicle and scrotum of rabbits by inoculation. Nichols showed that the lesions produced in rabbits might be milder than those of syphilis are more apt to be multiple and are more easily cured.

The disease is not venereal and infection in man occurs most commonly through abrasions in the skin by direct contact with an individual afflicted with yaws. Direct transmission of the virus from a yaws lesion to an open wound of the skin may occur also through insects especially flies.

Flies are generally recognized as potent factors in the spread of the disease the avidity with which they feed on yaws lesions having frequently been noted.

Kumm (1935) has brought forth evidence which suggests that in Jamaica the minute fly *Hippelates pallipes* of the family Oscinidae may

lesions of yaws subside rapidly while those of syphilis become indurated by new formation of fibrous tissue. In the earliest lesions of both syphilis and yaws excised (one week after inoculation) the spirochaetes were located in the epidermis and related structures the hair follicles. In the syphilitic lesions the organisms were also seen in the adjacent dermis and in about small blood vessels. The multiplication of *T. pertenue* appeared to be arrested early and the inflammation reaction produced was slight. *T. pallidum* however continued to multiply in both epidermis and dermis spreading deep into the tissues accompanied by an infiltration of lymphocytes and other cells.

In the larger lesions of syphilis necrosis and an exudation of polymorphonuclear leucocytes are prominent and spirochaetes are numerous. In the lesions of yaws there are few spirochaetes and they disappear rapidly. There is no evidence of phagocytosis of spirochaetes by any type of cell in either yaws or syphilis.

Turner (1937) concludes (1) that *T. pallidum* possesses pathogenic properties which differ from those of *T. pertenue* and (2) that the differences noted between yaws and syphilis in man are due in part at least, to inherent differences in the causative agent of each disease. Thirteen strains of yaws spirochaetes studied gave rise to the same type of lesions in rabbits and each of 8 strains of syphilis spirochaetes recovered from persons living in Jamaica produced lesions which while similar to each other were readily distinguishable from those produced by yaws spirochaetes.

The treponema of syphilis as contrasted with the treponema of yaws may be referred to as panblastotropic, with a tendency to invade and multiply in all tissues and produce lesions in them but it is especially mesoblastic in its tendencies (Schöbl). Syphilitic lesions are found for example in the skin the mucous membranes the bones the muscles and the viscera, as well as in the nervous system. The organism may also invade the cardiovascular system and the placenta, giving rise to congenital syphilis. On the other hand the treponema of yaws may be designated as epiblastotropic in its tendencies, invading and producing lesions as a rule in only certain tissues, particularly the skin and later the bones. Usually the internal organs the nervous and cardiovascular systems are not invaded and the disease is not congenital. In a few instances lesions of the internal organs and of the cardiovascular system have been reported in yaws. However syphilis has not been rigidly excluded in many such cases.

**Epidemiology**—Human and animal experiments show the ready transference of the disease to a second animal by inoculation in which the initial lesion makes its appearance in about three weeks, to be followed in man by a generalized eruption a few weeks later.

Yaws shows a striking limitation to the tropics and the effect of climate upon yaws is seen in the fact that it is so limited and does not spread in temperate climates from cases occasionally introduced. Also it is more common at low altitudes and in areas with higher rainfall in the tropics. In the colder climate of the mountains in some tropical countries there is a tendency for the cutaneous lesions to become condylomatous and limited and to occur particularly about the genital perineal and axillary regions. Thus Ramsey noted that in the cold climate of the mountains

cent of those infected were Cuban born and 59 per cent of these Cubans were negroes. Kinell (1944) has reported a case in a white man in the South Pacific. He was American borne of Italian parents.

**Immunity**—In man as well as in animals it has been demonstrated by experimental inoculation that one attack of yaws frequently confers protection against a second attack. Turner (1936) confirmed these facts by human experiments. Immunity to reinoculation of heterologous strains of yaws spirochaetes develops slowly during the course of the natural disease and seems to develop more slowly than in syphilis. Within the first 3 years reinoculation may give rise to a modified attack of yaws but after a period of 10 years the majority of yaws infected persons are refractory to reinoculation. Schobl has called attention to the possibility of exciting immunity in early cases of treponematosus disease by the use of treponema vaccines. He suggests that the inoculation with treponema antigen during the early stages of exaggerated tissue reactivity will accelerate the onset of immunity and prevent occurrence of late lesions of yaws and that such inoculations could be used also as a preventive measure.

There is much convincing evidence that an individual who has acquired an immunity to yaws is also immune to syphilis and vice versa. In Guam naval medical officers repeatedly have noted the striking immunity to syphilis of the natives almost all of whom have had yaws in childhood and Daniels observed the same fact in Fiji. Hudson (1936) described in the desert Arabs of Syria a form of syphilis (Bejel) largely acquired recently in childhood which closely resembles yaws except for the absence of a primary yaw but found no venereal syphilis among them. The latter disease however was prevalent among the Arabs in the towns. Butler (1939) suggests Bejel is congenital syphilis. Stitt (1941) points out that a striking difference between the two treponematoses yaws and bejel is the great frequency of oral mucous patches in bejel and the infrequency in yaws.

**Relation to Syphilis**—The exact relationship of yaws to syphilis is still a matter of controversy. Chambers (1937) points out that it is generally accepted that yaws is not hereditary and that it does not give rise to any congenital stigmata corresponding to Hutchinson's teeth, bossing of frontal bones, iritis, etc. Those who maintain that the diseases are distinct point out other differences in clinical manifestations and in the lesions in experimental animals which have been discussed. They also have emphasized: (1) The usual absence of mucous patches in yaws. Carter however found no mucous patches in 231 American negroes with syphilis although he found 21 cases among an equal number of white patients. (2) The rarity of tabes and paresis in yaws. These conditions are also rare in syphilis among primitive peoples in the tropics. (3) The reported absence of typical aortitis. Recent observations however show that aortitis may not be uncommon and Carl Weller (1936) from a study of the aortas of 169 cases in Haiti, a majority of whom had had yaws, concluded that yaws and syphilis produce identical lesions. But it has been thought by some that some of these cases had syphilis. As mentioned Takahasi (1937) who has infected rabbits with strains of syphilis and yaws found changes in the aorta in 168 rabbits infected with syphilis but none in 13 infected with yaws or in 3 with rat bite fever. In the syphilitic rabbits necrosis of the media and inflammation



be an actual carrier of the organism of yaws. He has observed enormous numbers of these flies on the ulcerative lesions and has found *Spirochaeta pertenuis* in the anterior gut or oesophageal diverticulum or stomach of the fly. The spirochaetes survive for about 7 hours in the diverticulum and it is suggested that the yaws infection is transmitted to another individual by regurgitation of an infected "vomit drop" when the fly feeds upon any abrasions of the skin or ulcerations which may be present. Krumm and Turner (1936) showed that rabbits could be infected with yaws by scarifying the skin and exposing them to *Hippelates pollipes* which had fed on infective discharges. These flies were observed in swarms feeding on the discharges from yaws lesions. Over 1500 flies were caught feeding on yaws sores within 15 minutes. In Jamaica the curve of incidence of new cases parallels the rainfall and (consequently) the prevalence of *Hippelates*.

Thompson and Lamborn (1934) in Africa, have shown that non biting haematophagous muscids feed readily to repletion on blood serum, serous exudate, ulcers, sores, and also secretions from the nose, eyes and mouth. After a meal a certain proportion of these flies pass blood or serum in their numerous dejecta which may contain large numbers of protozoa or the *Treponema pertenuis* of yaws.

These flies can infect any breach of the surface of the skin either through their dejecta or by regurgitation.

*Treponema pertenuis* of yaws was shown to pass rapidly in a viable form through the gut of *Musca spectanda*, and so could easily be deposited on cuts and abrasions.

**Age Distribution**—In the West Indies and Ceylon about two thirds of the cases occur before puberty although no age is entirely exempt. Turner and Saunders (1935) report that in Jamaica among more than 1800 persons who had had yaws, the disease was acquired before the age of 15 years in over 90 per cent. Saunders and Muench (1937) found in Jamaica in a yaws infected rural area that the proportion of the population with yaws increased rapidly to a level of 70-80 per cent at 15-20 years of age after which there was a gradual decrease. The peak of the infection rate occurred at about 8 years when about 20-30 per cent of the people were infected. This was followed by a more gradual fall. They found few new infections after 30. However, Parto Castillo (1938), in Cuba where the disease is comparatively rare found that 14 per cent of the cases were over 40 years of age.

The disease is much more common in males than in females. In some localities from 2 to 3 times as many males as females are infected.

**Race**—Another feature to be noted in the epidemiology of yaws is the vastly greater susceptibility of colored races, even those of mixed white blood showing a certain degree of immunity. In fact, it is almost exclusively confined to the colored races. Also there appears to be a predilection for certain native races. On the whole the negro and negro stock is especially liable to attack. In Cuba where Castillo (1938) reports the disease is limited to a single province Oriente 97 per

usually observed. In contrast to syphilis there is usually not the perivascular cellular infiltration which is seen in the corium in that disease.

A common late lesion of yaws consists of gummatous ulceration of the skin and subcutaneous tissue. The histologic study of these ulcerative lesions shows that the pathological process is characterized by the presence of granulation tissue in which there are richly cellular areas and proliferation of the fibrous tissue with very few if any giant cells. The inflammatory process is usually of rather a diffuse character and not sharply limited in nodular form as it so often is in syphilis. The cellular

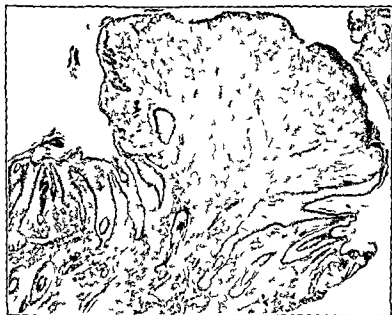


FIG 89.—As is to be though a yaws nodular form thus as shown in Fig 92. Note the position of granulation tissue with the knowledge of the intercapillary pectus and a nodule containing well developed epithelial cell polymorphonuclear cells and granular debris. X 30 (Army Medical Museum photo No 46953)

exudate is composed particularly of plasma cells, endothelial cells, fibroblasts, polymorphonuclear leucocytes and small round cells. In a few instances nodular areas are present in the center of which coagulation necrosis has sometimes occurred. This central area is surrounded by proliferating fibroblasts and round cells, and this zone in turn is surrounded by an area of granulomatous tissue which is richly vascular and contains many round cells, endothelial cells, plasma cells and a few polymorphonuclear leucocytes. Eosinophils are not prevalent or noticeably increased in number in the tissue. The vessel walls show in some areas moderate endarteritis and thickening of the walls and evidence of periarteritis with proliferating endothelial cells extending into the surrounding tissue.

of the media and adventitia were observed and aneurism was frequent. In a number of the human cases with aortic lesions the possibility of syphilitic infection could not be entirely excluded (Chandler, 1940). Hazen (1936) has emphasized the frequency of annular lesions and condylomata in the syphilis of the American negro. The annular or circinate type of lesion however has been noted in yaws (ringworm yaws).

Nevertheless the morphological identity of the organisms, their common serological relationships, and the response to the same therapy as well as the general similarity of many of the experimental lesions and many of the clinical manifestations indicate that the diseases are evidently very closely related. For years in numerous articles and in his monograph on syphilis (1936), C. S. Butler has insisted that yaws is syphilis modified by race, climatic influences, immunity, extragenital infection in childhood and absence of specific treatment. He has made a very careful and prolonged study of this question and a number of his views have been concurred in by many during the past few years. Butler and Peterson have suggested the term *treponematosis* as including yaws and syphilis. One must admit that from a clinical standpoint the term *treponematosis* or *treponemiasis* is especially convenient for the tertiary lesions of yaws and syphilis, which cannot often be distinguished except sometimes by the history of the case and the history is very frequently a very doubtful point among many of the peoples who commonly suffer with yaws.

### PATHOLOGY

The cutaneous lesions of yaws are papules which soon become eroded and moist and exude a yellowish secretion which dries into a crust. On removal of the crust a superficial excoriation with clean cut edges is seen lined with granulation tissue which bleeds readily. On examination of a section under the microscope elongated papillae may often be seen in the base of the excoriation or superficial ulcer, sometimes almost reaching the surface. Frequently there is hyperplasia and thickening of the inter-papillary pegs and below polymorphonuclear cells and a dense infiltration with plasma cells. Usually the *Treponema pertenue* is found only in the epidermis but occasionally it may be demonstrated in the perivascular tissues, in certain of the elongated papillae.

A striking feature of the yaws lesion is the great thickening of the epidermis and the degenerations which occur in the epithelial cells. In the later stages there may be hyperkeratosis. Much of the thickening of the epidermis is due to serous exudate and leucocytic infiltration. In the epidermis the leucocytes are often grouped in circular masses as in milium abscesses or they may be scattered diffusely throughout the epidermis. The elongated papillae are vascular, are frequently infiltrated with lymphocytes and leucocytes and often show small haemorrhages in the corium particularly in the deeper portions. Plasma cells are very numerous and constitute the great majority of the infiltrating cells though lymphocytes and a moderate increase in fibroblasts are

Stitt (1929) has pointed out that the main point in the pathology of a yaws lesion is the predominating involvement of the epidermis and the comparatively slight change in the corium. In a Levaditi stained specimen the spirochaetes are found in the epidermal layers instead of in the corium as with syphilis.

**Visceral Lesions**—It is usually stated that yaws confines itself to the skin even failing to invade the mucous membranes. Involvement of mucous membranes may sometimes occur through direct spread from the skin in contrast to common primary mucous lesions in syphilis. Noel has reported involvements of the nasal, buccal and conjunctival mucous membranes in yaws and there are many descriptions of invasion of mucous

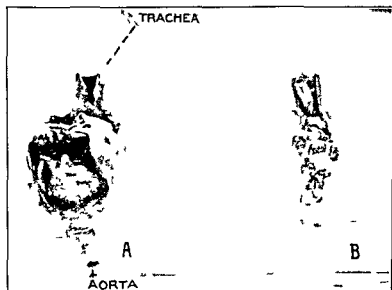


FIG 9.—Aorta and yaws from two cases diagnosed tertiary yaws. (A) The condition (B) blood vessel (Chamberlain)

membranes in the gangosa lesions of tertiary yaws. In Schob's monkeys the nasal mucosa was invaded by extension of the process down the skin surface of the nose. Acheson in 11 cases, 3 in adults and 8 in children, noted lesions completely on the mucous membrane of the lip.

Among the Fijians Harper has reported circulatory and central nervous system involvement. Lambert in the Fiji Islands after emphasizing that syphilis is almost unknown there reported that 42 had died of general paralysis of the insane and he thinks many of these probably had yaws in childhood. In a series of 526 consecutive autopsies at the Haitian General Hospital Choisser was unable to differentiate the visceral lesions of yaws from syphilis and made the diagnosis or recognized the lesions as those of yaws from the clinical history. He thinks the first evidence of

Marked evidence of extensive endarteritis and thickening of the vessel wall so characteristic of syphilis, is not present. Rather surprising is the slight tendency in the ulcerative lesions to the secondary invasion of the tissues by spirochaetes and fusiform bacilli and by cocci. Spirochaetes have not been found in the corium of any of the cases studied. In some instances it is only by the histological study that the lesions can be differentiated from those of syphilis. However, in other cases it is not possible to distinguish the well advanced tertiary lesions of yaws from those of syphilis and hence a few observers classify all such lesions including those of the viscera and cardiovascular system under the term *treponematosis*.



FIG. 90.—The aortas from two cases diagnosed tertiary yaws showing marked aortitis (Choi ser.)

Williams (1935), who has carefully compared the pathology of yaws and syphilitic lesions also concludes that the *late* ulcers of yaws and those of syphilis are so much alike that a diagnosis between them is frequently impossible. The differences in the histological picture are not marked enough to be decisive in many if not most cases. Ferris and Turner (1938) also think that in many cases the histologic criteria for the distinction of the cutaneous and subcutaneous lesions of yaws and syphilis are in general unreliable. However Williams believes that the evidence that has been presented to show that aneurysms of the aorta are caused by yaws appears unconvincing and that it is probable that the internal viscera in general are not involved in yaws, though the autopsy evidence upon this point is insufficient.

However Manson Bahr (1935) points out that no visceral changes have been found peculiar to yaws although *T. pertenue* has been encountered in the spleen lymphatic glands and bone marrow. He emphasizes that the important point of contrast in the morbid anatomy of yaws and of syphilis is the absence of endarteritis in the former and its frequency in the latter.

#### SYMPTOMATOLOGY

The native with yaws rarely presents himself at a clinic until the skin lesions are abundant or the joint or bone pains cause him to seek relief. The efficacy of arsenicals and bismuth preparations in treatment has



FIG 93—Typical general eruption in African child (Havard E. p. dit. 1930)

probably tended to change this attitude but even now the old fear in some localities handed down for centuries of the striking in of the disease may cause the patient to hesitate to subject himself to a treatment which will cause the mother yaw to disappear as if by magic. It was a custom of the slaves in the West Indies to inoculate children having but few lesions in other parts of the body in order to multiply the lesions and thereby to lessen the seriousness of the after course. In this way a superinfection was produced. In earlier years tertiary lesions of yaws were not recognized and some of the lesions that are today reported as tertiary manifestations of yaws were frequently referred to as syphilis. Before the recognition and acceptance as yaws sequelae of the disabling and mutilating conditions of the later periods there was little objection to

yaws damage may show itself in a degeneration of the intima of the aorta with yellowish patches of atheromatous change, the lesions being as a rule about 2 mm above the aortic cusps but not appearing to invade the valve itself. The atheromatous change may extend down to the iliac bifurcation. He believed aneurysms were extremely common in late yaws, all varieties being encountered and that there was a tendency to early rupture. The epicardium was more or less opalescent with porce



FIG 92.—Yaws—usually described type of frambeside. (Army Medical Museum photo No 39197)

lain like patches. These areas were also frequently seen in the endocardium. In the liver Choisser frequently found small superficial punctate scars which showed on section areas of degeneration with associated round cell infiltration. Actual cirrhosis was rare. Gummata of the liver also were rare but did occur. Haemorrhages into the brain and cord were common especially in young adults. Weller (1936) also believed the lesions of the aorta may be identical in yaws and syphilis.

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### SYMPTOMATOLOGY

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FIG. 93.—Typical eruption of yaws in child (Harold Expedition 1930)

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noted which often abate upon the appearance of the initial papule at the site of inoculation. There may be enlargement and tenderness of the tributary lymphatic glands about the time of the appearance of the eruption. This initial lesion may be single or there may be several papules grouped together. In some cases it may be impossible to get any history of a primary lesion or it may have been overlooked. The primary lesion



FIG 95—Yaws—ma 1 papule framboesia (A my Med 1 M um ph 1 No 39 (4))

is almost invariably extragenital and it has the same appearance as the lesions of the secondary stage thus differing from syphilis.

The yaws lesion whether primary or secondary starts as a papule which in a few days enlarges to the size of a small pea. It is conical and surrounded by an inflammatory areola. At this time the thickened epidermis begins to crack and a yellowish seropurulent fluid exudes from the underlying fungoid base. It bleeds easily but is not painful. It is this fungoid yellowish or yellowish red tubercle which has been thought

the division of the clinical course into the primary stage and the secondary one. With the designation of these late manifestations as representing a tertiary stage, the objection was raised by some that such a division confused the separation of yaws from syphilis. If the dualist insists on a different terminology the division of yaws into the initial stage, the generalized stage, and the sequelae might be regarded as acceptable. Sellards and Goodpasture are convinced that in many cases yaws termi-



FIG. 94.—Yaws—c reinate frambo side (Choussier)

nates spontaneously with the secondary stage while in other cases it lies latent, or proceeds to tertiary manifestations.

**The Initial Stage**—The native child without clothing and with frequent skin wounds furnishes favorable opportunities for the introduction of yaws virus either by contact with an infected child or with fomites or insects which have been feeding on the yaws of other children.

During a period of incubation, averaging 3 or 4 weeks, vague digestive troubles, nocturnal headache, joint pains and an irregular fever may be

organs 12 However the usual statistics fail to give more than one or 2 per cent of lesions of the genital organs

According to the Jamaica Yaws Commission (Saunders Turner Johnston 1935) in approximately 75 per cent of the persons in Jamaica with yaws mostly children the initial lesion occurred on the lower legs and feet Very commonly in children it occurred about the mouth rarely on the anus or prepuce

In their ordinary locations the yaws tubercles are not painful unless pressed firmly but when located on the palms of the hands or soles of the

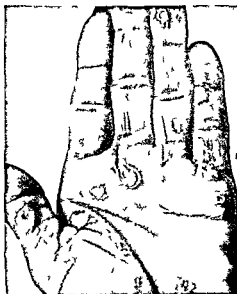


FIG 97—Pallary yaws e a th m f the p lm of th h nd th months f e b g n  
ng of th d (Aft Muhl n )

feet the thick skin of these regions exerts pressure so that in such situations the lesions are painful

In some cases itching of the papules may be troublesome The rule is for the secondary lesions to resemble the primary one yet in tead a symmetrically distributed papular eruption much like that of syphilis has been reported by some as a manifestation of yaws Some have reported dry scaly patches scattered irregularly over the body as preceding the more characteristic granulomas These desquamating patches may disappear and reappear in the course of successive eruptions In this stage a generalized painless enlargement of the inguinal glands has been described much as in syphilis The generalized stage lasts from 3 or 4 months to 2 or 3 years the yaws tubercles coming out in successive crops in long standin<sub>g</sub> cases

to resemble a raspberry hence the name framboesia. French authors liken it to a fig which has been turned inside out. The papule or papules may continue to enlarge until there is present a cauliflower like mass 1 or 2 inches in diameter covered by a moist dark honey comb like crust. The single tubercles are generally round or ovoid and vary greatly in size averaging one centimeter in width by four millimeters elevation. The developed primary lesion has sometimes been termed the 'mother yaw'.

*The Generalized Stage*—In from 6 weeks to 3 months after the appearance of the initial lesion which may have dried up and left only a scar



FIG. 96.—Characteristic lichen-like eruption in framboesia. (After Mayer.)

or which more commonly is still present there again set in malaise, headache and joint pains with or without an irregular fever.

The secondary eruption is made up of lesions having the same character and course as the primary yaws tubercle. In the general eruption the papules appear not infrequently in the region of the junction of skin and mucous membrane as about mouth, nose and anus. In such regions they may become very moist and resemble the condylomata of syphilis.

Besides their location on face and about the perineal region they are numerous on neck, arms, legs and buttocks. They are rare on the trunk and scalp. Noel gives as percentage of sites of the initial lesion: Lower extremities 46, upper extremities 18, face 12, trunk 12 and genital

from 10 to 11 weeks and the lesions were similar to those of the initial one. Interesting was the fact that one of the 6 cases failed to show an initial lesion following the first and second inoculations but developed macules on the palms and papules on the hairy part of the neck which contained *treponemata*.

The Wassermann reaction was positive 2 or 3 weeks after the appearance of the primary lesion and increased rapidly in titre. No fever was noted in any of the cases. The glands tributary to the primary granuloma enlarged first and were followed by a generalized enlargement—this being greater than that generally noted in syphilis.

**The Sequelae or Tertiary Lesions of Yaws**—Daniels noted in the Fiji Islands destructive lesions of the naso-pharyngeal region which he thought



FIG 99.—Papilloma of the foot. C b yaws. (After S. Haff.)

might be associated with a preceding yaws attack. He noted cutaneous lesions which resembled lupus vulgaris. Boissiere has noted not only the nasopharyngeal lesions and lupus vulgaris like ones but also tibial involvement, joint swellings and dactylitis.

Numa Rat described various tertiary manifestations. There may be subcutaneous nodules about ankle or leg which soften and may produce bone lesions and deformities. He noted destructive lesions of nares, pharynx and palate which may set in years after an attack of yaws. His description of the process starting as an ozoena or sore throat followed by destruction of the uvula, velum palati and septum nasi is much like that of gangosa. Howard has noted the greater frequency of destructive lesions of the nasopharynx in those parts of Africa where yaws is prevalent than in parts where syphilis prevails.

*Peculiar Types of Yaws*—When yaws tubercles develop in the palms of the hands or soles of the feet we have a very painful and incapacitating condition resulting. The pressure of the thick unyielding epidermis on the tubercles beneath gives rise to marked pain, thus differing from tubercles on other parts of the body. Eventually these tubercles break through and the affected sole may have a worm eaten appearance. The name "crab yaws" has been applied to such a condition involving the soles of the feet and was so called from the difficulty in walking which was said to resemble the locomotion of a crab. In some cases the yaws tubercles adjoin one another to form a circle enclosing unaffected skin. Such



FIGS. 98.—Thickening of ulna above wrist and end of left tibia. Ngord (treponema) (Harvard African Expedition)

an arrangement of lesions has been described under the name of "ring worm yaws."

*Inoculative Lesions*—To study superinfection in yaws Sellards, Lacy and Schobl inoculated six volunteers and in five cases obtained a sharply outlined granuloma about 5 cm in diameter and about 3.5 mm elevation. The color was reddish and the appearance lobular. There was erosion but never ulceration excluding secondary infections. These primary lesions, yielding treponemata, appeared in from 3 and one half to 4 weeks after inoculation. The reinoculations were made about 1 week after the appearance of the granuloma of the first inoculation and there resulted at the site of the succeeding inoculation papules or granulomas similar to those of the primary one. The secondary eruption appeared in

Patients with the disease have rarely been observed prior to the full development of the mutilating ulcerations. In a few cases however it was noted that the mucous membrane involvement occurred from the adjacent skin lesions or that a patch of membrane appeared in the region of the soft palate. This membrane rapidly became honeycombed and an examination 3 or 4 days later showed underneath a deep ulcer surrounded by an area of marked congestion.



FIG. 101.—Gang. (Army Medical Museum phot. N. 39206.)

The ulcerating process usually advances rapidly destroying bone as well as soft parts. The process seems to extend from within outward giving rise to a funnel shaped loss of tissue. The ulceration advances upward and forward eventually destroying the nasal septum and structures forming the tip of the nose leaving the upper lip as the lower border of this external opening. As a rule the larynx is not affected. The nasal duct seems to be prone to attack and it is through this channel that the process may reach the eye to bring about destructive tendencies in that organ.



According to Castellani the characteristic lesions of tertiary yaws are gummatous nodules and deep ulcerations.

In many cases, the lesions persist as chronic ulcerations which may invade the neighboring tissues extensively. Shattuck (1930) has studied particularly Ngonde, a name applied locally in parts of Central Africa to tertiary lesions of yaws which are characterized by scarring and ulcerations of the skin and subcutaneous tissues. Such lesions were especially observed where the bone lies close beneath the skin, e.g., on the forehead, face, joints of the shoulders, elbows, wrists, lower legs, ankles and feet. Periostitis, joint lesions and dactylitis were frequently seen.

In recent years joint and bone lesions have been more frequently reported as complications of yaws than formerly. In addition to a form of multiple dactylitis, a yaws onychia is well recognized. Maul has estimated that about 20 per cent of the cases of yaws in the Philippines show



FIG. 100.—Yaws. Oteopriostitis of fingers. (From Joyeu after Cl. Mouchet.)

bone or joint sequelae. The X-ray examinations bring out rarefied areas of bone, most of which appear to start within the bone, although at times the process starts peripherally.

Periosteal nodes, similar to those of syphilis, occur not infrequently on the anterior surfaces of the long bones or the forehead. An osteitis may result in a sabre shin, or produce deformities of the arms or fingers. Chesterman (1930) has found anterior posterior bowing of the tibia as one of the commonest bony lesions of tertiary yaws in the Congo. Synovitis of yaws origin may give joint changes similar to those of syphilis.

Among other tertiary manifestations of yaws may be mentioned gangosa, juxta articular nodes and goundou, heretofore classed as separate diseases.

**Gangosa (*Rhinopharyngitis Mutilans*)**—A Spanish commission in 1928 investigating the diseases of the Ladrone Islands separated gangosa (which, in Spanish means muffled voice) from leprosy. The condition is now well recognized as existing in those parts of the world where yaws is prevalent.

**Goundou**—This condition has been reported from various parts of the world where yaws is common especially Central Africa South America and Jamaica In 1882 note was made of the presence of the horned men among natives of the West Coast of Africa Another native name is anakhre Many views as to its etiology have been advanced but its following so closely upon secondary yaws often commencing in quite young children and the fact that goundou victims have an immunity to heavy inoculations with yaws virus and that along with the development of goundou go the other tertiary lesions of yaws all of which can be controlled in the early stages by arsphenamine make it reasonable to place at least many cases of goundou in the category of sequelae of yaws

Along with persistent headache and a thin purulent discharge often tinged with blood the enlargement of the nasal processes of the upper maxilla proceeds in a downward and outward direction Osteocopic pains worse at night are frequent The shape is generally oval and by involving other bones of the face a projecting tumor as large as the first may finally result The overlying skin is normal and is not attached to the bone As the exostoses grow larger the nasal passages are obstructed and later on vision is interfered with by obstruction of the line of vision The growth does not tend to invade the orbits

Botreau Roussel gives the following statistics as to the frequency of locations of exostoses in tertiary yaws paranasal 121 other tumors of the superior maxillary 23 inferior maxillary 16 skull 2 tibia 69 fibula 5 femur 4 radius 5 and clavicle 3

Goundou is usually bilateral but may be confined to one side and at times there may be supplementary tumors

Not all investigators believe that goundou represents a tertiary lesion of yaws Indeed in some instances an apparently similar condition has occurred in cases where yaws has apparently been absent A similar disease has been reported in the higher apes chimpanzees and baboons and Letulle has found characteristic lesions of goundou in an Inca skull from Peru Manson Bahr (1940) points out that there is very little difference between the bony changes of goundou and those found in *Leontiasis ossea* Stannus has pointed out that hyperostosis of the facial bones has been observed in Paget's disease *Osteitis deformans* Some observers have suggested that goundou is more akin to *Osteitis fibrosa* due to interference with the bone metabolism and an endocrine disorder and that the yaws infection may constitute the nonspecific factor in under nourished native children There is a possibility that goundou may represent a form of *Leontiasis ossea* occurring in certain tribes of people who are afflicted at the same time with yaws

Treatment consists in the surgical removal of the outgrowths and in the intravenous and intramuscular injections of neosalvarsan According to Botreau Roussel 4 or more injections are necessary before improvement is observed He has operated with success upon 113 out of 130 cases which he studied in the Ivory Coast

Gangosa is common in Dominica West Indies where 60 cases in a population of 2000 were observed, also in Guam, the Carolines Fiji British Guiana West Africa and the Belgian Congo. It is rare in children and young adults, though Leyes reports that in Guam he has observed it in children.

It is often impossible to say whether the lesions of gangosa represent a late manifestation of yaws or of syphilis. The majority of those who have recently written upon the condition have expressed the opinion that it is a tertiary manifestation of yaws. Such an opinion has probably in some instances been given particularly on account of the fact that yaws in childhood prevails in those districts where gangosa is also found and where generally genital syphilis is not observed to be common. The writer studied histologically a case in which the lesions did not suggest leprosy, nor did they especially suggest syphilis particularly in the absence of marked vascular changes. Modifications more or less characteristic of

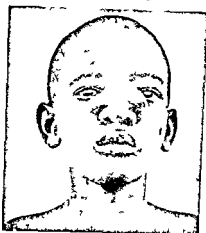


FIG 102—Gangosa. (From Friedrichsen after May r)

yaws were present but all that could definitely be stated was that the lesions gave evidence in many respects of a chronic inflammatory condition in which there was a tendency to destruction of newly formed tissue with little tendency to invasion by microorganisms from the air or from the surface of the skin. Cocci were found on the surface of the suppurating lesions. Neither spirochaetes nor fusiform bacilli nor leprosy bacilli were discovered. Hallenberger who has recently examined the condition from the pathological histological standpoint believes that *Rhinopharyngitis mutilans* belongs to the syphilis framboesia group of lesions and in the absence of the classical inflammatory changes of the vessels which are present in syphilis he regards it as a late lesion of framboesia.

Gangosa is obviously a condition which occurs only in localities where proper treatment is not available or among ignorant unhygienic people who neglect treatment.

Secondary infection with different microorganisms invariably plays a role in modifying more or less the appearance of the lesions of gangosa as it does in other ulcerative lesions of the mucous membranes of the nose, mouth and throat. In more recent years a number of observers have sometimes attributed the condition to leishmania infection. Treatment of these advanced lesions is usually unsatisfactory, but the process may sometimes be arrested by repeated injections of neosalvarsan.

A few cases of juxta articular nodules have been found in individuals who have not been outside of Europe and at least one not outside the United States. Nevertheless the disease is said to be rare in white people and only about 25 cases in the white race have been reported.

Webber, Goodman and Young and Worster Drought have reported somewhat similar lesions under the name subcutaneous fibroid syphilomas and Patane, Akovbian, Da Fonseca and Jessner have observed juxta articular nodules in cases of late syphilis. Araujo says that he has found no cases associated with yaws but has observed 60 cases in syphilitics. Hu and Frazier have reported *Treponema pallidum* in their cases. Monacelli and Pisani have reported upon 3 brothers all children with amyotrophic lateral sclerosis associated with juxta articular nodules which were regarded as the result of hereditary syphilis. Jessner in a study of the literature accepts 62 cases of juxta articular nodules in which he believes yaws can be excluded as a cause. Twenty of these were from Europe and North America and 42 from North Africa including 3 cases of his own. He not only found no spirochaetes in the lesions but rabbit inoculations proved negative. Mendelssohn was unable to infect monkeys with material from the nodules.

Other recent observers besides Mendelssohn report neither the association of yaws nor of syphilis. Among these are Kadaner and Aramaki. The latter points out that no cases of juxta articular nodules have been seen in Japanese who had never left their own country with the exception of the one case he reports. Fourteen previous cases in Japanese had been reported from Palau Island. In Aramaki's case there was no history of syphilis or yaws and the Wassermann reaction was negative. Joyeux and Jeanselme have also excluded yaws and syphilis in the cases they recently have reported.

The tumor masses vary in size up to that of a golf ball and are very hard in consistence. The skin over them is at first freely moveable but later on may become attached. They are located subcutaneously especially about the external surfaces of the extremities and particularly in relation to the joints. They are not sensitive and rarely or never suppurate. The course is most chronic and but rarely do they become absorbed. Study of the literature reveals that it is quite obvious that juxta articular nodules often result about localities which are frequently subjected to pressure and to light blows or bruises often repeated. Under certain conditions they may result from different forms of mechanical irritation just as other true neoplasms may sometimes do and in some instances they may be onchocercal in origin and in others framboesial or syphilitic.

In the last instance syphilis or yaws may act as a predisposing factor among people or races with especial tendency or diathesis to the abnormal proliferation of fibrous tissue. However it is difficult to explain the peculiar inflammatory lesions by such a hypothesis alone. Also while racial tendencies may be a partial factor in their production it should be borne in mind that although these lesions are very common in parts of Africa they do occur in many other parts of the tropical world. There

**Juxta-articular Nodes**—These are nodes composed of irregularly distributed bands of poorly vascularized connective tissue without elastic fibres. There may be necrotic areas and irregularly distributed polynuclear cells. It has been stated that in syphilis these nodes are more frequent about the joints of the upper extremities, while in yaws the location is more apt to be on the legs.

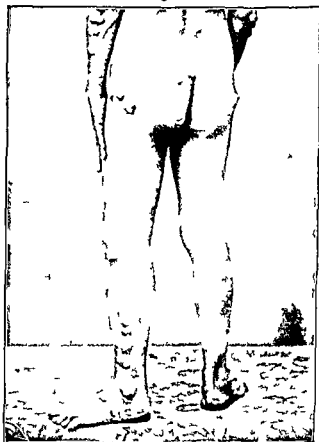


FIG 103 —Juxta art icular nodules (After Steine )

The nodules have a varied etiology. Onchocercal infection not infrequently gives rise to nodules in the vicinity of joints. Before the yaws or syphilis etiology of at least one type of these nodules was generally accepted by a number of investigators, it was thought that the cause might lie in various fungi, especially *Discomyces carougeau*. However, such etiology has not been confirmed.

A number of investigators have failed to find spirochaetes in the nodules. However, Van Dijke of the Dutch East Indies and Sobernheim in Berne, and Clapier in Africa, as well as Van Hoof, have reported the presence of spirochaetes, each in one instance.

(1) *The initial lesion of syphilis is generally located on the genital organs while that of yaws is extra-genital.* However the chance of innocent syphilis is extragenital and as yaws is innocently acquired there may be agreement in location.

(2) *The difference between the Hunterian chancre with its induration and the framboesoma with its lack of induration and friable crust.* Stokes emphasizes the impossibility of diagnosing the initial lesion of syphilis on clinical grounds—we have to depend on the dark field search for spirochaetes and the Wassermann reaction. This also is true for yaws.

(3) *Glandular enlargement more pronounced in syphilis.* In his inoculation of volunteers with yaws Sellards reported greater glandular enlargement than might be expected in syphilis.

(4) *Absence of mucous membrane lesions in yaws and the presence in syphilis.* Noel has reported such lesions as occurring in the nose, pharynx and conjunctiva of African yaws cases.

(5) *Absence of visceral lesion in yaws.* There is no agreement of opinion on this point. In those cases in which visceral lesions have been observed at autopsy some observers believe syphilis has not been rigidly excluded in the diagnosis and that also infection with both diseases may occur. In the careful and routine autopsies conducted in the Haitian General Hospital Choissier has found aneurysms and other arterial degenerations to be as common in cases he regarded as yaws (from the history) as in syphilis. Cerebral haemorrhage in young persons who have had yaws was reported as a frequent cause of death. Pathological changes in liver and heart were also noted.

(6) *Absence of tertiary lesions in yaws.* For the past 10 years the tertiary lesions of yaws have been accepted as occurring and as being possibly more disabling than those of syphilis. Formerly these sequelae were classed as separate diseases.

(7) *Absence of general paresis and tabes in yaws.* This statement is denied by some of the most competent and experienced observers, as Harper in the Fiji islands. At any rate even if we accept this distinction the same in a measure holds true for syphilis when prevalent in primitive people. It has been suggested that the exemption is connected with lack of treatment and we know that some psychiatrists are concerned over the possibility that modern anaesthetics may have increased the number of parasyphilitics. It has been claimed that freedom from these treponematous cerebral affections is connected with the great prevalence of malaria in the tropics. While this possibly may be a factor we know that the absence of tabes and general paresis is recorded in some yaws regions which are free from malaria. Another suggestion is that having the same embryological origin if the brunt of the attack is borne by the skin the central nervous system is spared. In articles on tabes and general paresis we often find the statement of the absence of any history of primary sore or secondary lesions. Stitt points out that his study of the records of cases of general paresis in the Navy would indicate that of 57 such cases no evidence could be obtained of either a primary or secondary eruption in 7.

(8) *Equality of the Wassermann reaction of the cerebrospinal fluid.* The examination of the cerebrospinal fluid with the Wassermann reaction in yaws has generally been found negative [Fischer, Turner, Saunders and Johnston, Fernando (1938)] while in syphilis the cerebrospinal fluid usually gives a positive Wassermann reaction.

Slamet Sudibyo (1939) has made a comparative study of the cerebrospinal fluid in yaws and syphilis. In the cases of yaws no changes in the fluid were found after the third year of the disease. In 123 syphilitic cases changes were found in all stages of the disease as usually found in syphilis with increased cell count. In the first in 83 per cent in the secondary stage 35.7 per cent in the tertiary stage 42.8 per cent and in the latent stage 0.7 per cent. In the cerebrospinal fluid of 61 yaws cases changes were found in the secondary stage in only 14 per cent while no changes were found in the latent and tertiary stage. Also the changes were not so pronounced as those in syphilis and were considered a passing reaction of the cerebrospinal fluid from the general yaws infection. From his investigations he concluded that no definite evidence can be presented of the development of central nervous system lesions in yaws which resemble the tabes dorsalis and general paralysis of syphilis.

is nothing entirely pathognomonic about the tumors. The changes found in one type encountered by the writer consisted especially of a dense fibrous tissue capsule enclosing inflammatory and necrotic areas in which peculiar large swollen lipoid cells with multiple inclusions in their protoplasm were situated. These appearances distinguish and separate this form of nodule very strikingly from that of onchocercal origin. This type of lesion was originally described by Jeanselme.

In those parts of Africa where the tumors due to *Onchocerca volvulus* are found there may be confusion in diagnosis but the filarial nodes are more often elastic. By aspirating the swelling microfilariae should be found in onchocerciasis.

The treatment of juxta articular nodules is by excision should they give trouble.

### DIAGNOSIS OF YAWS

**Clinical Diagnosis**—Lesions of cutaneous leishmaniasis and blastomycoses which may be confused with the naso pharyngeal and other tertiary lesions of yaws can be surely differentiated only by finding the respective causative organisms. The differential diagnosis between syphilis and yaws may be difficult. Chandler points out there is no characteristic primary lesion in yaws as in syphilis and usually mild constitutional symptoms appear 2 or 3 weeks after infection. There is no rash as there is in syphilis and prenatal infections never occur. The characteristic feature of the disease is the development of one or repeated crops of raspberry like tumors on the skin crusted over by dirty, yellow cheesy caps. Tertiary lesions of skin joints or bones may occur but according to some observers the viscera eyes and nervous system are not involved. The secondary framboesal lesions of yaws are seldom imitated by syphilis. However it is impossible to differentiate the causative organisms and the serum in both diseases gives a positive Wassermann reaction. There is overwhelming evidence that when an immunity exists for yaws there is an immunity for syphilis and on this ground we can explain the failure of the natives of yaws countries to become infected with syphilis. Nevertheless as Manson Bahr (1940) points out both diseases may concur in the same individual and antecedent syphilis certainly does not confer absolute immunity to yaws nor antecedent yaws absolute immunity to syphilis. Several observers have observed yaws and syphilis simultaneously in the same patient and Carman (1935) a case of simultaneous infection with yaws and primary syphilis. Yaws may die out in a community yet syphilis remain. Yaws may be universal in a community as in the Fijians and Samoans and yet true syphilis whether as an acquired or as a congenital disease be unknown. Manson Bahr also emphasizes that in yaws Hutchinson's famous syphilitic triad the characteristic notched teeth nerve deafness and interstitial keratitis are absent.

Other points of distinction which have been debated and suggested have been the following

gangosa Halton obtained 37.3 per cent positive Wassermann reactions Maltaner (1941) found 41 of 44 sera from yaws patients reacted in the quantitative complement fixation test for syphilis Kerr found that 73.8 per cent of 2429 natives of Guam had had yaws usually in childhood

Among other diseases which may be confused with yaws particularly as regards the nasopharyngeal ulcerations of tertiary yaws may be mentioned American cutaneous leishmaniasis The differentiation rests in finding *Leishmania tropica* in such lesions The ulcerations of leprosy and tuberculosis may sometimes be confused with the lesions of yaws The examination for acid fast bacilli may furnish means for differentiation

Bromide eruptions may in some instances greatly resemble yaws but the history of the taking of the drug and the effect upon withdrawal should differentiate

### PROPHYLAXIS AND TREATMENT

**Prophylaxis**—Prophylaxis depends especially upon the adoption of measures to prevent infection by direct contact with yaws cases There should be isolation and segregation of the afflicted and prompt treatment by salvarsan (arsphenamine) In an endemic district especially care should be taken to prevent flies from having access to abrasion of the skin All cuts or sores should be protected by a dressing Care should also be taken to prevent articles of clothing contaminated with yaws discharges from acting as infecting agents

**Treatment**—There is no more striking example in medicine of the specificity of a drug than that of arsphenamine (salvarsan) or neoarsphenamine in the treatment of yaws As the writer first showed in 1910 salvarsan has a marvelous curative effect during the framboesial stage of the disease the lesions rapidly disappearing in the course of a few days Arsphenamine or neoarsphenamine should be given in the same manner and with the same care as in the treatment of syphilis and preferably intravenously to adults and if possible to children except when very young For adults the intravenous dose of neoarsphenamine advocated is 0.6–0.9 gm For children up to 10 years of age 0.3 gm but under 2 years 0.1 gm However in yaws excellent results are obtained by intramuscular injection into the buttocks of 0.4 gm dissolved in oil Also intramuscular injections are preferable in very young children

In the field in connection with mass treatment it is frequently much more convenient to give intramuscular than intravenous injections

Usually within 10 days time the granulomata of the skin are absorbed and disappear Frequently one dose has effected a cure when given early in the disease but in order to prevent relapses 2 or 3 doses are advisable Especially in cases where much bone destruction has occurred repeated injections are often required Some cases are very resistant and those with tertiary lesions frequently require prolonged treatment

In the Philippines using 0.01 gram of neoarsphenamine per kilo weight of patient with 2 treatments as the rule but occasionally including a third one clinical cures resulted in 94.3 per cent of cases In Samoa it was



Pardo Castello has studied in Cuba the cerebro spinal fluid in 25 cases of yaws, in which the stage of the disease is not mentioned. In 13 there was excessive value of globulin in 3 pleocytosis with a maximum of 11 lymphocytes. In some of the cases the Lange gold curve resembled that of syphilis. Stannus states that such changes resemble those that have been found by other observers in some cases of yaws. In 6 of Castello's cases the Kahn test was found to be positive.

David (1938) examined 27 cases of yaws. With one exception uncomplicated yaws did not give a positive Kahn reaction.

Gutierrez et al. have examined the cerebrospinal fluid in 145 cases of yaws especially selected to exclude syphilis. In none was the Wassermann reaction positive. The cell count in all 122 cases was normal. The Pandy test was slightly positive in 1 of 116 cases examined. Total protein was normal in 53 cases in which estimations were made. The colloidal gold test was similarly negative in 29 cases tested. The total 145 comprised 2 cases of primary, 42 secondary and 101 of tertiary yaws. Stannus (1942) points out that in yaws there is some evidence that a temporary reaction in the cerebrospinal fluid may occur.

**Absence of Cardiac Disease**—Turner, Saunders and Johnston (1936) in the careful study of the disease in Jamaica record that no cardiac disease has been encountered in yaws cases and this they have confirmed by radiographic examinations. They report the attack rate in adults to be as great as in children and yet nothing resembling congenital syphilis was observed in Jamaican babies. This they hold is strong evidence against the identity of syphilis and yaws. All the female cases gave a definite history of having contracted the disease from their children, who were infected with yaws.

The fact that yaws is not hereditary and that no case of congenital yaws has been observed is generally admitted.

It would be possible to continue the discussion of the so called points of distinction between yaws and syphilis further but it should be apparent from the discussion already given that the exact relationship of yaws to syphilis is still controversial.

Attention has already been called to the views of Williams also held by other observers to the effect that the evidence that aneurysms of the aorta are caused by yaws appears unconvincing and that syphilis has not been excluded in such cases. Chandler (1940) also believes that the viscera, eye and nervous system are never involved in yaws. It has already been noted that no changes in the aorta of rabbits infected with yaws have been found. Chambers (1938) believes firmly that yaws and syphilis are distinct infections and gives an excellent differential table. A study by Turner (1937) of yaws among persons living in rural Jamaica and of syphilis among persons living in Baltimore showed easily recognizable differences between the two diseases.

**Laboratory Diagnosis**—Staining of the juice expressed from yaws tubercles by the Indian ink method or with Giemsa's stain is the usual procedure advised for detection of the spirochaete. However the examination of such material with the dark field is often found to be of greater value. Sections from a yaws tubercle treated and sectioned according to Levaditi's method may show the treponemata in the region of the thickened interpapillary pegs of the epidermis.

The Kahn test which has been the official serological test for syphilis in the United States Navy has been found quite as reliable as the Wassermann reaction as an aid in the diagnosis of yaws and has the outstanding feature of relative simplicity of technique. Baermann gives the percentage of positive Wassermann reactions in untreated clinically positive cases as 80-100 per cent in treated cases 50 per cent and in latent ones as from 35-40 per cent. In an examination of the serum of 281 cases of

Fitzgerald and Gupta found that bismuth salicylate alone was of little value but in combination with neosalvarsan it was most useful

A great advantage of the bismuth drugs is their low cost as compared to the arsenicals. Their great disadvantage is that they cause greater pain than sulpharsphenamine does at the point of injection and often cause a distressing stomatitis. These features may discourage the patients from receiving a sufficient amount of treatment. Also bismuth is less efficient than the arsenicals and more injections are required to bring about the same results. When conditions make the administration of neoarsphenamine practicable it is certainly to be preferred to sulpharsphenamine because of the comparative painlessness of the intravenous method and the consequent encouragement of patients to continue treatment. The same principles of contraindications for arsenicals in yaws hold as in syphilis. Renal disease, aneurysm and individual idiosyncracies when present must be considered in the choice of a drug and its dosage and the same measures in regard to the treatment of reactions are applicable as in the treatment of syphilis. For arsenic elimination sodium thiosulphate is of value.

Potassium iodide is of great value as in the treatment of syphilis and it seems to be particularly indicated in cases suffering from the headaches and rheumatic pains which are so common in very late yaws. DeLangen believes that with tertiary yaws potassium iodide is often of value. Many large ulcers of several years' duration will not completely heal after extensive arsenic and bismuth and local treatment. In many such cases the healing is greatly enhanced by weekly intravenous injections of a 1 per cent solution of tartar emetic, the initial dose being 5 cc. This may be increased to 10 or 15 cc. later if it is tolerated. Sometimes the ulcers are found to contain great numbers of insect larvae. These are readily removed after the application of chloroform.

The leg ulcers must receive daily cleansing and dressing and when possible the member affected must be put at rest. A suspension of 1 per cent iodoform in ether dropped onto the ulcer daily by means of a medicine dropper gives the ulcer surface a fine coating of iodoform which repels insects and conceals the offensive odor of necrotic tissue. Deformities and a variety of crippling conditions resulting from periostitis, contractures and extensive ulcerative and cicatricial changes may require plastic surgery for relief.

In the treatment of the lesions of the soles of the feet termed *bubul* in Netherlands India local treatment is usually necessary. If the epidermis is too much hardened it may best be softened by immersion in a warm weak solution of washing soda. Afterwards the skin is shaved off until the papillomata are exposed and then touched with copper sulphate crystals. Later an ointment is applied. DeLangen has found that the action of salvarsan alone is not so apparent in the treatment of some of the cases of *bubul*.

Aneurysm may require when occurring in an extremity the ligation of arteries. Yaws lesions seem to respond to antiluetic drugs even more rapidly and gratifyingly than syphilitic lesions do.

**Rural and Mass Treatment**—Yaws often occurs especially among very poor natives living in rural and often remote districts and its question of treatment is not only related to the individual but is frequently a public health measure. Consequently the cost of drugs and those which can be readily administered to large numbers of patients if need be at points of considerable distance from clinical centers must be considered.

found advisable to give 3 injections of 0.6 gm of neosalvarsan at weekly intervals for an adult male and smaller doses for women and children

Morse in Santa Domingo, treated 1064 cases of yaws with salvarsan. Five years later he revisited the country and examined again nearly half the cases previously treated. About half had been free of yaws signs for 5 years but about 46 per cent remained uncured. He found that after treatment with 3 injections a cure was likely to be permanent. The only objection that has been raised to the use of arsphenamine or neoarsphenamine for mass treatment of natives is its cost.

Circular 56 Surgeon General's Office United States Army November 1942 states the preferred arsenical is Mapharsen. Adult dose for males is 0.06 gram and for females 0.04 gram. The following standard course of treatment for yaws is recommended: 4 weekly injections of mapharsen or neoarsphenamine and bismuth subsalicylate given on the same day. This is to be followed without a rest period by 4 weekly injections of mapharsen or neoarsphenamine alone which in turn is to be followed by 8 weekly injections of bismuth subsalicylate alone. Take serologic test if possible with eighth and sixteenth treatment. Follow the patient by clinical examination and serologic tests at monthly intervals for 3 months and then at intervals of 3 months for one year. If a clinical relapse occurs or if the serologic test remains positive for 6 months after treatment has been started repeat course of treatment outlined above.

**Bismuth Preparations**—Most authorities concur in the belief that none of the many bismuth preparations are equal in efficiency to neoarsphenamine. However bismuth preparations have been shown to be of value and superior to mercury and potassium iodide for the treatment of yaws.

Sodium potassium bismuth tartrate has been the salt generally used and it should contain from 50 to 60 per cent of metallic bismuth. It should be injected intramuscularly in oil suspension in the dosage of from 0.15 to 0.3 gm. The injection should be repeated in about 7 days. The toxic manifestations of bismuth are similar to those of mercury so that we should be on guard for stomatitis and evidences of renal irritation. On account of stomatitis and albuminuria, diarrhoea and skin rashes which have been observed especially in the Solomon Islands and the Congo treatment with bismuth has been less satisfactory. Sometimes there has been considerable induration and abscess formation may result in the tissues as a result of the injections. There are various proprietary bismuth preparations but the factor of cost enters into the use of these products so that there would be no advantage over arsphenamine. Combined neo-bismuth treatment has also been recommended.

Levaditi has reported favorably on the spirochaeticidal effects of 3 of these bismuth preparations viz bistovol bismuth arsanilate and bismuth tryparsamide. Bistovol is a precipitate resulting from the action of sodium potassium bismuth tartrate on sodium stovarsol. This like stovarsol can be given intramuscularly or by mouth and in syphilis of the rabbit excellent results have been reported. The experiments showed it to have a curative action in doses of 5 milligrams per kilo.

Bismuth arsanilate (Bi O<sub>4</sub> 53 per cent arsenic 17 per cent) has seemed to show an effect equal to that of bistovol. It is given intramuscularly suspended in oil and has an advantage over bistovol in that it is almost painless when injected into man.

Bismuth salicylate and precipitated metallic bismuth in oil are also in use. The dose of each of these drugs is 0.1 to 0.2 gram in 10 per cent solution (or suspension especially in olive oil). In a small series of cases in Haiti Turner Saunders and Johnston (1935) found the results of treatment with neoarsphenamine were considerably superior to those with bismuth salicylate or halarsol though in some cases the results with bismuth were excellent.

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The bismuth preparations and sulpharsphenamine both of which may be given intramuscularly, seem to be the drugs of choice for rural work. Sulpharsphenamine is used in doses of 0.1 gm for infants to 0.4 gm for large adults. The solution is prepared by using 1.0 cc of sterile distilled water to each 0.4 gm of sulpharsphenamine and is injected into the buttock muscles at weekly intervals. One or 2 doses may heal the early lesions but later lesions may require continuation of weekly injections over periods of many weeks or months with the usual rest periods as in the treatment of syphilis. The bismuth preparation which has most commonly been used is sodium potassium tartro bismuthate in aqueous solution.

*Stovarsol* (icetarstone) — Stovarsol, which can be given by mouth is obviously a more convenient method for the treatment of large numbers of natives in the field than injections of arsphenamine. It is advised to begin with 1.0 gm daily increasing to 1.5 to 2 or 3 gms on successive days for adults and 0.5 to 1.0 gm for children. Chesterman has found that 3 doses only frequently effected a cure. He has pointed out that he can give in stovarsol 10 times the corresponding dose of neosalvarsan. Van den Branden reports that after a total amount of from 8–15 gms of this arsenical compound had been given the Wassermann reaction became negative. Slight diarrhoea has been the only untoward symptom sometimes observed. The full course of treatment with stovarsol may be more expensive than the course of the injections with neoarsphenamine.

*Carbarsone* (p carbamino phenyl arsonic acid) also given by the mouth has also been recommended. It is given in the same doses as stovarsol. The therapeutic dose recommended is 75 mg per kilo body weight.

*Halarsol* (oxyameno phenyl dichlorarsine) in the form of intravenous and intramuscular injections in doses of 0.125 to 0.25 gm for 3 doses at intervals of 3 or 4 days has recently been employed in the Congo with good results. The minimum toxic dose is stated to be 4.5 mg per kilo weight.

The natives afflicted with yaws are frequently suffering also from malaria and from intestinal parasites. Treatment of these diseases should also be given as it obviously may aid materially in improving the general health and promote more rapid recovery from yaws. Frequently the question of nutrition is of the utmost importance and patients who are debilitated from defective nutrition may show remarkable improvement of their yaws lesions when admitted to institutions where they receive proper nourishment.

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subsequent extension to the backs of the hands and in some cases to the arms and legs. Saenz regarded keratosis of the palms and soles as an outstanding characteristic of the Cuban cases. In a study of 50 cases in Cuba only one showed pigmentary disturbances on the face and one disturbance over the abdomen and thighs. These were the sole exceptions. However in Mexico and Colombia keratoses were not observed in patients with pinta and dyschromic changes of the palms or soles were reported as absent or very rare. Later (July 1940) Leon y Blanco reported that he found cases in Mexico with plantar and palmar keratoses. Also no keratotic or peeling lesions were observed by the Commission which studied the affection in Colombia in 1940.

**Geographical Distribution**—The disease has been very prevalent in Mexico and Colombia where it has reached endemic proportions. Saenz (1940) in addition mentions its occurrence in Cuba, Venezuela, Brazil, Peru, Central America and the West Indies (Haiti, the Dominican Republic and Guadelupe). Leon (1940) has reported upon its prevalence in Ecuador and Souza Araujo (1940) observed cases in Ecuador and Argentina. He stated however that in Brazil mal del pinto or carate had not been observed although he thinks that the disease may exist in Brazil amongst the inhabitants in the high Amazonas region in the boundaries along Colombia and Peru. He also mentions that in Uruguay the disease is not known to exist. The prevalence of the disease in Mexico was emphasized by the Mexican Commission in 1929-31 which found that 11 per cent were affected of 2 500 000 people examined. In Colombia it has been said that 4 per cent of the people of some districts suffer with the disease. Iriarte in 1932 reported some 400 000 cases were present in Colombia. In 1941 he states that in Venezuela the average for the whole state of Barinas is 10%.

#### ETIOLOGY

For many years the disease has been regarded as a parasitic one and caused by fungi. Manson Bahr (1940) describes it as an epiphytic disease and points out that if one of the scales is moistened with liquor potassii and placed under the microscope black spores and a wide highly refractory mycelium are found and he illustrates and describes this fungus. Montoya y Flore (1898) especially emphasized and described different species of fungi as the cause of the affection and reported that various chromogenic fungi caused the different shades of color in pinta. Castellani confirmed these ideas. Some observers have reported the isolation of *Aspergillus* and *Penicillium* from the scrapings but according to others the most common fungus found was *Dematiium uerneckii*. Brumpt (1936) has given a list of 27 species of fungi reported to be the cause by various authors. However a number of recent observers have regarded the fungi obtained from the scrapings of the lesions as common saprophytes. Fox (1940) has believed for 10 years that fungi play no part in the etiology and it has seemed evident for some time that the old idea that differently colored lesions of pinta were caused by specific fungi is no longer tenable. In fact the different colors in the lesions seem to be

## Chapter XII

### PINTA

**Synonyms** —Carate (Colombia), mal del pinto (Mexico), azul (Chile) boussarole (Haiti) guassorolle (Dominican Republic), and numerous other colloquial names

**Classification** —Since the last edition of this text book our conception of pinta and its etiology has greatly changed so that it can no longer be properly classified among the diseases of the skin caused by fungi. More over recent studies seem to demonstrate that it now should be regarded like yaws as a form of treponematosi. Admiral Stutt has recently emphasized the importance of the new investigations regarding pinta and has made a study of the recent literature upon the subject. The present article has been prepared almost entirely from his reports and notes and the new literature relating to the disease.

There have been suggestions that carate existed among the Aztecs at the time of the Spanish conquest and that Cortez established a sanitarium believing that this disease was a form of leprosy. Oviedo referred to certain individuals who shed their skin as carates. The term pinta is apparently derived from the Spanish term *pintado* (mottled or painted) or from the word *pinta* (a spot). In English text books the term pinta has been employed but in Spanish publications the term *mal del pinto* is used.

Holcomb (1942) has given a most complete review of the literature regarding this disease which should be consulted by all interested in its history and the progress of its study.

**Definition** —Pinta is a disease which becomes manifest by the development of dyschromic changes in patches of the skin. These more or less depigmented lesions may be numerous minute and discrete, or confluent and much larger and assume a lead or slate blue color, or more rarely a pinkish tinge. In other cases yellow or violet white or black patches have been noted. The spots of altered skin are often first noticed about the wrists or backs of the hands more rarely on the face or other exposed portions of the body as the arms feet or legs. If portions of the scalp are affected the hair may turn white in such areas. Healed or arrested lesions of pinta may closely resemble or be indistinguishable from vitiligo. In Mexico the lesions have been symmetrical in about one third of the cases. The Wassermann reaction has been found positive in the later stage of the infection in almost all of the cases. Keratoses of the palms of the hands and soles of the feet have also been observed.

In the classical descriptions of the disease and in more recent reports by Castellani (1919) Brumpt (1936) Fox (1939), Manson Bahr (1940) and others it has been pointed out that the palms of the hands and soles of the feet are not affected. However Saenz and his assistants (1940) who have studied the affection in Cuba reported that pinta in its primary stage is limited exclusively to the palms of the hands and soles with

to find the *Treponema* before might have been due to the fact that he examined pintids (a name given hyperchromic lesions by Leon y Blanco corresponding to the term syphilids). It is in the early papules that the *Treponema* can most readily be found. In February 1939 Iriate in Caracas, Venezuela, also reported the presence of the spirochaete in persons with carate. Brumpt (March 11, 1939) announced to the Societe de Biologie, Paris, the discovery of this organism by the Cuban investigators and proposed the name of *Treponema carateum* for it. Leon (1940) also reported the finding of the *Treponema* in cases in Ecuador.

Gomez (1942) has performed dark field examinations in 500 cases and in 98% the spirochaete was found.

**Morphology**—The measurements of the *Treponema* as well as its appearance by dark field are similar to those of *Treponema pallidum*. Iriate and the other members of the Venezuela Commission noted that its motility decreased about 20 minutes after the specimen was taken. Leon y Blanco (1940) gives the average length of  $17.8\mu$  from 500 measurements. Ordinarily it measures from 12 to  $18\mu$ . The number of spirals changes according to the length of the particular specimen observed. It is readily stained by the common silver impregnation methods advised for staining spirochaetes in smear preparations and also by Ciemsa's stain. He reports that a 10 per cent solution of saponin dissolved the organism in 6 hours at room temperature. The same result was obtained either by sodium taurocholate or bile. Varela and Roaro (1940) report that the organism dies in a much shorter time in bile than does the *Treponema* of syphilis.

Blanco has not succeeded in cultivating the organism and reports that a suitable animal for inoculation still remains undiscovered.

**Animal Inoculations**—Saenz (1940) states that the cornea and testicles of rabbits were inoculated with fragments of tissue from persons with pinta and that keratitis and epididymitis similar to the lesions obtained in experimental syphilis resulted. However, he gives no details of such experiments. The Venezuela Commission report that the inoculations in guinea pigs, rabbit and rat were negative.

**Human Inoculations**—Herreyon (1938) reported negative results obtained by Mooser and Varela in Mexico by inoculation of healthy men and laboratory animals with blood or exudates from the lesions of pinta. However, Leon y Blanco (1940) has demonstrated by inoculations into a series of 28 volunteers including himself that the disease is infectious and may be easily transmitted when a small amount of serum from a pinta lesion is inoculated. The series of volunteers included 4 groups: (1) 17 normal individuals lacking any evidence of syphilis or pinta and negative serologically; (2) 3 syphilitics with positive serology (Wassermann reactions), one of these having had a chancre 3 years previously, one infected 14 months before inoculation and one with a history of chancre 4 months before the experiment; (3) 5 pinta cases with pintids, treponemata and positive serology. All the normal volunteers except 3 in which the serum was deposited on the intact skin developed a primary papule going on to a secondary eruption. The 3 syphilitics also developed an initial papule but the secondary eruption was scant and atypical while of the volunteers with pinta one showed a characteristic take and the other 4 gave negative results. Generally a papule developed within 3 weeks fol-



connected especially with varying degrees of atrophy of the derma which eventually results in white spots similar to vitiligo. Also the varying colors are probably related to some extent to environmental factors.

Chavarría and Shipley (1925) reported that the Wassermann tests in cases of carate were generally negative. However, Menk (1936) found that the reaction was present in pinta in 74.5 per cent. The following year the Mexican Pinta Commission, directed by Herrejon, reported the Wassermann reaction present in nearly 100 per cent of the cases. Also they failed to confirm the idea that fungi were the cause. Moreover the fact that the blue patches of the affection responded to antisyphilitic treatment strengthened the suggestion of a spirochaetal causation.

In 1927 Herrejon suggested that the disease was produced by a *Treponema* related to that of syphilis or to pian (yaws) and Fox (1930)



FIG 104.—*Treponema carateum* (*Treponema herrejon*) (Photomicrographs of Dr. León y Blanco)

also thought the condition might be a general infection of a spirochaetal nature. In August 1938 Armenteros and Triana in Cuba observed a *Treponema* in lymph obtained by abrading the epidermis at the edge of a palmar lesion in a case of pinta. Blanco in December 1938 in the same case found the *Treponema* in material obtained by puncture of a lymph gland the preparation being stained by Levaditi's method. To this organism the name of *T. herrejon* was later (May 1939) given in honor of Dr. Herrejon of Mexico. Saenz Armenteros and Triana reporting in 1940 also found the *Treponema* in August 1938 in microscopical preparations made from the lesions and the following year also in other cases. However, Saenz (1940) reports that staining of sections of the tissues for spirochaetes gave positive results only twice and failure in 30 instances. Even in cases in which the spirochaetes were detected by dark field the results were negative in the tissue.

The presence of the *Treponema* in the pinta lesions was confirmed in Mexico by Blanco in October 1938 in 7 of 8 cases. Later it was found in 89 of 100 cases studied. It has been suggested that the failure of Herrejon

specific causative agent *Treponema herrejoni* since identical primary lesions follow after inoculation of infective material from these different types of spots. The *Treponema* he states was detected in every case 254 times in 254 cases examined in the primary lesion. He states that the

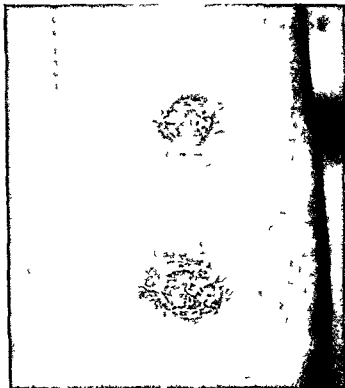


FIG. 105.—Int. le p. m. nt. l. n. th. erythem. t. qu. mous stage 65 d. y. fte. the e. p. r. m. nt. l. inoculat. n. Th. le. n. n. et. th. l. bow. w. obt. n. d. w. th. ult. n. f. Me. an. materi. l. The. m. d. st. nt. n. with. a. t. pon. ma. from. Cub. r. case. (C. of Dr. L. ny. B. l. an. o.)

primary lesion of pinta is different from that of syphilis or yaws in that it is always a closed lesion of papular appearance and does not become ulcerated. The primary lesion always develops at the site of entrance of the *Treponema* both in the experimental and he believes in the naturally acquired disease. He found that carate could be transmitted experimentally from individual to individual by using virulent material from any clinical type of lesion and no matter in which stage of the disease a sample of lymph was taken and he points out that hence an intermediate host for the transmission of the disease is not necessary. Also the experimental inoculations showed that syphilis does not render the patient immune to pinta.

lowing the experimental inoculation of some of the serum taken from the lesion of an individual suffering from pinta. Secondary lesions sometimes in crops developed later.

Blanco found that positive (Wassermann) reactions were not obtained in the inoculated cases prior to the development of the secondary eruption.

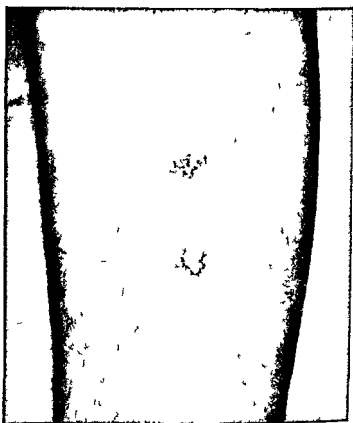


FIG. 105.—Initial experimental lesion in the erythematous squamous stage. The lesion situated nearest the elbow was obtained with the Mexican strain of treponema. The lesion most distant from the elbow was obtained with the Cuban strain. The lesions developed in an identical manner and hence it was concluded that the Cuban and Mexican strains were identical. The photograph was taken 37 days after the experimental inoculation. (Case of Dr. Leon y Blanco.)

(about 60 per cent) and only in the advanced cases with marked pigmentation did the positive tests approach 100 per cent. From these observations it has been suggested that immunity develops much more slowly than it does for yaws and hence considerably later than it does for syphilis.

Blanco summarizes his experiments with the statement that the pigmentary leucodermic and erythematous pigmentary spots almost constantly observed in cases of pinta or carate are only produced by one

did not show any treponemata. It has been suggested that other insects may be concerned in transmission but there is no evidence to this effect. Some observers have not found *Simulium* abundant in some regions where pinta is common. Iriarte (1944) in studying the disease in Venezuela thinks the insect vector is *S. exiguum*.

Blanco points out that if an insect vector exists it might transmit the disease by depositing upon the abraded skin its faeces containing the *Treponema* or by being crushed upon the abraded skin at the time of biting. In 3 experiments in which the infected material was placed upon the intact skin the results were negative. However when a drop of infected tissue lymph was placed on a slight erosion of the skin made with a pin infection followed.

### CLINICAL MANIFESTATIONS

Pinta to judge from descriptions generally found in text books has been frequently confused with other affections of the skin. Many of the cases of pinta the writer observed years ago were in Buena Ventura or nearby regions in Colombia where a large proportion of the inhabitants suffered with skin diseases of which carate was by far the commonest. The disease occurred particularly among the lower classe of people who were obviously not accustomed to washing and bathing and to the use of soap and whose skins were dirty and variable in color sometimes with an offensive odor. Apparently many examinations of the skin for the presence of fungi and other microorganisms have often been made without proper cleansing and disinfection of the surface of the skin of such cases.

It has been generally assumed that the primary patch occurs on some exposed portion of the body and that it first becomes hyperpigmented through active pigment formation later assuming a white red blue or black tinge gradually increasing in size and becoming scaly and itchy. The cases have often been divided according to the color of the individual lesions and types of the disease have been described as red yellow white blue black and violet. Other patches often noted have been of leaden hue. In fact these have received the name of *plombo* resembling spots caused by massage with mercurial ointment. Some earlier writers as Scheube likened the appearance of some of the cases to a painted circus clown. Other spots of coffee color or white have more recently been regarded as representing later atrophic or cicatricial changes.

The Mexican Commission (1929-31) which found 270 685 cases in 15 states affected listed the color of the lesions as occurring in order of frequency the blue type the white the mixed the lead colored the violet the black the red and the yellow. Distribution was symmetric in one third. Scaling was present in one third and itching in one fourth. Some authors report that it is exceptional to find an individual with a single type of spot. Almost always there is an association of colors as white blue coffee-colored and red. The spots vary much in size. In many cases they measure 2-4 mm in diameter when they are usually multiple and

## EPIDEMIOLOGY

Pinta is favored by a moist, warm climate and it prevails along the banks of streams in the valleys of tropical or sub tropical countries. The disease occurs especially with isotherms of 80°F and above. Moisture seems more important for the persistence of both yaws and pinta in the tropics than any other factor. Indeed yaws almost disappeared in Jamaica during a record drought, from 1838 to 1848.

The usual method of transmission is not definitely known. Most authors agree today that the disease is not contagious and that it is not hereditary. However in regard to hereditary infection cases have been observed in infants 1-5 years of age and it has been suggested that in some of these transplacental infection may have occurred and the disease not become manifest until some time after birth. However, there is no evidence to support this suggestion.

According to statistics published by Gonzalez Uruena of the Mexican government (1934) for 27 685 cases of mal del pinto observed in 15 states of Mexico, the following ages were given of the infected individuals: 0-1 yr 707 cases, 1-5 yrs 6 803, 5-10 16,229, 10-20 33 686, 20-30 50 174, 30-40 60 487, 40-50 59 779, above 50 42 820 cases. However in certain regions where 11 593 cases of pinta were observed constituting about one fourth of the population no infections were demonstrated under 1 year of age and therefore it was thought that in the cases reported from 1-5 years in the statistics they have probably been towards the age of 5 years. Montoya y Flores in Colombia reported that the disease was very rare from 3-4 years of age.

According to other statistics in 254 cases the infection was most common between the ages of 6-10 years (81 per cent) next 15-20 years (67 per cent) and third 10-15 years (63 per cent). However, another report from Mexico stated that the disease was commonest in the 30-40 year decade.

**Transmission**—Leon y Blanco has demonstrated the causative *Treponema* in the discharges from fissures in the plantar hyperkeratoses of 29 of 41 cases. This highly infectious discharge might well serve as a source of infection through any abraded surface of the skin in another individual. Hence for the transmission of the disease an insect vector is not required. However in the discussion on yaws in Chapter V it has been shown that *Hippelates pallipes* may sometimes mechanically transmit yaws and it has been suggested that *Simulium haematopotum* may be concerned in the transmission of pinta.

Herrejon (1938) observed 48 *Simulium haematopotum* which fed on a case of pinta in which it was said a specimen from a lesion showed 8 treponemata to a microscopical field. In the ingested blood in four of the flies *Treponema* were found while in an equal number of control flies no *Treponema* were found. However the time they persisted in the flies is not stated and obviously no conclusions can be drawn from this experiment. *Pediculus humanus* and *Ornithodoros talaje* also fed

located on the buttocks when the mother had advanced lesions on the forearms

Blanco has divided the disease into three distinct stages showing different clinical serological and immunological features. The primary stage begins at the time of infection and lasts during the period when the initial lesion alone is present. This period was found to vary in different individuals being between 5 months and one year or perhaps even longer. The appearance of the primary lesion after it has become an erythematous squamous patch from which papules may appear



FIG. 08.—Initial lesion on the sole of the foot. The papules are covered with livid scales. Naturally acquired disease. (Case of Dr. L. O. Blanco)

peripherally may vary greatly. In some instances the lesion may be described as trichophytoid, psoriasiform, lichenoid, or large patches of other appearance may occur. The secondary stage is characterized by skin lesions or papules which rapidly change into diversely outlined erythematous squamous lesions for which the name of pintids has been proposed. This stage is reached after 5, 12, or more months have elapsed from the time of infection. The initial lesion continues to evolve during the secondary stage and becomes indistinguishable from the pintid eruption. In the tertiary or dischromic stage there are present achromic or pigmentary spots, erythema, follicular keratosis, keratoderma, and superficial atrophoderma. The serological tests are constantly negative while the initial lesion alone is present, that is during the first stage of the disease, and superinfection is always possible during this stage. In the secondary stage the Wassermann reaction is approximately positive in 60 per cent. The further the disease has progressed, the more likely is the reaction to be positive. Eosinophilia and an increase of the basophilic

scattered in groups over the skin. In other cases large, confluent, irregular patches are formed, as seen in the illustrations. The patches may be round, oval or irregular in outline. They are not elevated but are always strikingly visible, on careful inspection usually covered with fine scales but without desquamation.

In view of the studies carried on since 1938 it would appear that some of the earlier observations are erroneous. The evolution of the disease can perhaps best be understood from the study of 17 experimental inoculations reported by Leon y Blanco already referred to, and from his study



FIG 107—Erythema with squamous initial lesions after approximately 55 days (Case of Dr Leon y Blanco)

of some 39 additional cases of natural infection. According to the inoculation experiments the incubation period varies from 7–20 days by which time an initial papule appeared at the point of inoculation. This extended peripherally as a squamous erythematous patch reaching a diameter of about 1 cm in about a month and then continuing to spread peripherally. Secondary lesions appeared in crops around the primary lesion or elsewhere on the body in about 5 months (earlier or later). Progressive hyperpigmentation then occurred and later on depigmentation which gave rise to the various colors or vitiliginous like spots extending over the body. In naturally acquired infections the primary lesion is said to be almost invariably on the uncovered parts of the body particularly the legs to the sides of the feet and the arms to the hands and the face. Blanco has never observed an initial lesion on the palms of the hands or on the trunk but in an infant he has seen the primary papule

located on the buttocks when the mother had advanced lesion on the forearms

Blanco has divided the disease into three distinct stages showing different clinical serological and immunological features. The primary stage begins at the time of infection and lasts during the period when the initial lesion alone is present. This period was found to vary in different individuals being between 5 months and one year or perhaps even longer. The appearance of the primary lesion after it has become an erythematous squamous patch from which papules may appear



FIG. 108.—Initial lesion on the lower limb. The papules are covered with a thin layer of scales. Naturally the disease (Case of Dr. L. on y B. and o).

peripherally may vary greatly. In some instances the lesions may be described as trichophytoid psoriasisiform lichenoid or large patches of other appearance may occur. The secondary stage is characterized by skin lesions or papules which rapidly change into diversely outlined erythematous squamous lesions for which the name of pintids has been proposed. This stage is reached after 5, 12 or more months have elapsed from the time of infection. The initial lesion continues to evolve during the secondary stage and becomes indistinguishable from the pintid eruption. In the tertiary or dischromic stage there are present achromic or pigmentary spots, erythema, follicular keratosis, keratoderma and superficial atrophoderma. The serological tests are constantly negative while the initial lesion alone is present, that is during the first stage of the disease and superinfection is always possible during this stage. In the secondary stage the Wassermann reaction is approximately positive in 60 per cent. The further the disease has progressed the more likely is the reaction to be positive. Eosinophilia and an increase of the basophilic



leucocytes occur in a high percentage of the cases. Superinfection may also be produced by inoculation during this stage. During the tertiary stage of the disease, according to the figures of different authors, from 70-100 per cent of the cases give positive serological tests and experimental superinfections cannot be produced.



FIG. 109.—Areas of complete depigmentation of the hands and feet and a juxta-articular nodule in a patient with incomplete closed variety of pinta. (After Saenz. Courtesy Arch. Dermat. & Syph.)

Although Saenz (1940) apparently regarded hyperkeratosis of the palms of the hands and soles of the feet as primary manifestations of the disease, it would appear that these are really late manifestations of the affection, as similar lesions are in yaws. Saenz nevertheless believed that there was nothing to indicate framboesia in the 50 cases he studied and there were no morbid changes in the periosteum or bones, the lesions of pinta being confined to the epidermis and corium. In this connection it is interesting to recall that Hudson has observed patchy depigmentation of the skin in several cases of bejel in Arabia. He illustrates these in

one instance on the back and in another on the posterior surfaces of the hands and wrists

Neumann Moya and Brewster (1931) who have studied 75 cases by radiography in Colombia thought they were able to detect to some extent an aortic dilatation in 80 per cent and clinical signs of cardiac disturbances in 26 per cent of those under 30 years of age. Obviously in countries where syphilis prevails to a very great extent it is difficult to draw conclusions as to whether the pinta is uncomplicated by other *Treponema* infections. Saenz in 30 cases that he observed in Cuba also reported cardio aortic lesions such as aortitis aneurysm enlargement of the diameters of the heart and valvular conditions in 23.3 per cent. It hence is important to consider the relationship of pinta to the other forms of treponematoses yaws and syphilis.

**Histology**—In the studies that have been made by Leon y Blanco Herrejon and Saenz the histological examinations have shown treponemata to be chiefly located in the prickle cell layers of the lesions especially in the small areas of acanthosis in the epidermis where the granular debris resulting from the necrosis and a few leucocytes and treponemata may be observed by Levaditi's stain. A greater involvement of the corium has been reported for pinta than for yaws but much less than that which has been noted for syphilis. It may be recalled that Marshall in the Philippines (1907) emphasized that the chief histological differentiation of the yaws lesions from those of syphilis were represented in the degenerative changes in the epithelial layers and in the absence of periarterial and endarterial changes in the corium as noted in syphilis. Schüffner found spirochaetes only in the degenerative areas of the epidermis in yaws and never in perithelial relations as in syphilis. Stitt and others at the Naval Medical School Washington in the study of sections of yaws lesions confirmed Marshall's and Schüffner's results. These observations have since been substantiated by a number of other investigators.

Saenz (1940) found that in his study of pinta lesions staining of the tissues for *Treponema* gave positive results in only 2 cases in which the lesions were deeply infiltrated and failure in 30 cases. He points out that deeply infiltrated lesions were rarely observed. Blanco showed that the *Treponema* may sometimes be demonstrated in the gland juice expressed from the regional lymphatic glands in the lesions.

From the histological standpoint he describes the initial lesion as constituting a dermo epidermic papule. The following outstanding lesions were noted discrete keratosis acanthosis intercellular oedema showing small areas of spongiosis exocytosis and necrosis by the fusion of isolated cells of the malpighian layer. The dermis exhibits a very dense infiltration containing lymphocytes plasmocytes and scanty neutrophils and eosinophils melanophores and histiocytes are also present. This infiltration invades the papillary and reticular portions of the dermis and penetrates the deep portion of the dermis in the form of diffuse cellular invasions.

The hair follicles and the coiled portion of sweat glands are surrounded by layers of cells of the inflammatory infiltration.

leucocytes occur in a high percentage of the cases. Superinfection may also be produced by inoculation during this stage. During the tertiary stage of the disease according to the figures of different authors from 70-100 per cent of the cases give positive serological tests and experimental superinfections cannot be produced.



FIG. 109—Areas of complete depigmentation of the hands and feet and a juxta-articular nodule in a patient with complete crossed variety of pinta. (After Saenz. Courtesy Arch. Dermat. & Syph.)

Although Saenz (1940) apparently regarded hyperkeratosis of the palms of the hands and soles of the feet as primary manifestations of the disease it would appear that these are really late manifestations of the affection as similar lesions are in yaws. Saenz nevertheless believed that there was nothing to indicate framboesia in the 50 cases he studied and there were no morbid changes in the periosteum or bones the lesions of pinta being confined to the epidermis and corium. In this connection it is interesting to recall that Hudson has observed patchy depigmentation of the skin in several cases of "bejel" in Arabia. He illustrates these in

with such lesions suggested it and finally the discovery of a *Treponema* indistinguishable morphologically from *T. pallidum* confirmed it. Also Saenz in Cuba has reported a comparable incidence of arterial degeneration in such cases with pinta as has been reported by other observers in syphilis.

**Hyperkeratoses in Pinta and Yaws**—In view of the frequency with which hyperkeratosis of the palms and soles have been observed in pinta in Cuba it should be recalled that one of the most disabling and rather frequent conditions found in yaws is clavus or crab yaws. This is a hyperkeratosis of the soles of the feet. H. D. Chambers (1938) in his book on yaws states that spirochaetes have been found in the papular lesions of crab yaws which may come out alone or be associated with other tertiary lesions. In 1922 Moss and Bigelow reported the results of their study of yaws in the Dominican Republic. Of all the single manifestations of yaws clavus (crab yaws) was the most common. 327 cases had clavus alone present with no other sign of yaws while there were only 5 cases with palmar lesions which showed yaws hyperkeratoses as the only signs present. There were 579 cases with other evidence of yaws in addition to clavus and 68 cases with palmar keratoses associated with other lesions. The descriptions of these hyperkeratoses were similar to those given to the pinta plantar lesions. The response to arsenicals in these cases surprised the authors greatly. The moth eaten soles of the feet with their erosions and fissures began to desquamate and following the second injection to begin to return to normal healthy skin. P. D. Gutiérrez (1923) reported 431 cases of keratosis palmaris and plantaris in 658 yaws patients in the Philippines.

Saenz says that the descriptions of these keratoses correspond to those seen by him in Havana, Cuba. He also emphasizes that keratosis of the palms and soles has been an outstanding characteristic of the Cuban cases. Its first manifestation consists of rounded or irregularly outlined hyperpigmented spots which enlarge peripherally becoming at the same time more numerous. Newly formed areas also turn into keratoses which extend over the whole of the palms and soles. The skin appears dry and yellowish and becomes squamous at times when involvement is severe. Very rarely are hardened claviform keratoses seen. Fissures may develop interfering with manual work and walking. In the more advanced stage the pigment is destroyed with resulting permanent achromic areas. In 100 patients he examined in Mexico rudimentary keratosis of the central part of the hands was observed in two. According to the physicians in Mexico as has been noted above keratoses have not been observed in patients with pinta and dyschromic changes of the palms are also rare.

However in addition to the 2 cases with keratoses of the hands found by Saenz in Mexico Leon y Blanco (1940) in his recent studies of the affection in Mexico has also noted the occurrence of the palmar and plantar keratoses in that country.

Saenz in addition to the Wassermann reaction has also observed in 30 cases with palmar and plantar lesions cardio-aortic lesions such as aortitis aneurysm enlargement of the diameters of the heart and valvular conditions. Such disturbances were demonstrated in 23.3 per cent.

Just as in some other inflammatory skin conditions, the pigmentary function is affected in mal del pinto, scanty pigment granules may be seen inside the cells of the stratum germinativum. These pigment granules are also present in other cells of the malpighian layer and in the melanophores of the dermis.

The infiltration may enclose few elastic fibers, although such fibers exist in normal quantity in the rest of the dermis.

**General Considerations of History and Relationship**—In an editorial in the October 1939, number of the Bulletin of the Oficina Sanitaria Panamericana will be found an excellent summary of the history, epidemiology and distribution of this very important disease of a number of the tropical and subtropical regions of the New World. In this presentation twenty four different names are given for this disease and that number could be greatly increased were we to add the various local designations given by the natives of Mexico, Colombia, Ecuador, Venezuela, Cuba and other states of the tropical belt of the Pan American Union. The review again brings to our attention the importance of etiology in the knowledge of a disease entity as illustrated when Castellani first reported the finding of *Treponema pertenue* in yaws. Other investigators searching for treponemata in the lesions of numerous skin diseases in many countries of the tropical world established the fact that most of the affections in which treponemata were found could be grouped with the disease so prevalent among the African slaves brought to America and designated yaws or framboesia. Later on, the employment of the Wassermann test added to our means of recognition and brought out the additional relationship of these diseases to syphilis. In the many definitions of syphilis the designation protean is almost invariably included.

**Syphiloids and Bejel**—There have been described in the history of syphilis numerous syphiloids of which the better known are 'radesyge' of Scandinavian countries, sibbens of Scotland and "button scurvy" of Ireland. Various medical men of the West Indian Islands, in early Colonial days noted the resemblance between yaws and these European diseases. When Hudson with a long experience as a medical missionary among the Arabs of Syria reported a disease of the desert Arabs to which the name of 'bejel' was given by these natives and which showed both treponemata and positive serology but was essentially a disease contracted innocently in childhood as is true of yaws, the controversy which arose had a great value. The claims of Hasselmann who had only a few weeks to consider the nature and epidemiology of this disease as against the statements of Hudson who lived among these people for more than a decade are relatively unimportant as Hasselmann considered the disease as neither yaws nor bejel but plain syphilis. It may be stated that a striking difference between the two treponematoses, yaws and bejel is the great frequency of oral mucous patches in bejel and their infrequency in yaws.

Pinta or carate has been described as a disease essentially cutaneous. There was no idea that palmar or plantar keratoses bore any relationship to the disease until the occurrence of the Wassermann reaction in cases

Flavino Silva (1940) has described Pinta in Brazil where it is known as puru puru. It must not be confused with purru, a synonym of Yaws in Malaya.

Escobar has described Blue de matosis, a spirochetal disease somewhat resembling Pinta and observed in the inhabitants of the Chilean plateau. He thinks it should be differentiated from Carrate and that the organism is different from *S. carateum*. Some of the enlarged lymphatics contain fine grains of pigment.

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Changes were also found in the spinal fluid in 10 per cent. The changes consisted of an increased globulin content, a syphilitic colloidal gold curve and a positive Meinicke reaction. In one case repeated tests gave negative results in the blood but a positive one was obtained with the spinal fluid. In one case a juxta articular nodule was found at the lower part of the right leg.

Pardo Castello and Ferrer (1942) found the Complement Fixation and Precipitation tests positive in 60% of the patients in the early and 100% in the late stages. Of their 41 cases 52.1% showed changes in the spinal fluid similar to those of cerebrospinal syphilis.

Saenz says that in none of the 50 cases of pinta he has studied with these palmar and plantar keratoses was there anything to indicate framboesia or a previous history of it. The patients were all Cubans and none showed morbid changes in the periosteum or in the bones so commonly observed in the late stages of yaws. The lesions were confined to the epidermis and corium. The most important changes were in the horny layer and were manifested by pronounced hyperkeratosis. The thickness of the stratum corneum was increased sometimes to as much as 15 times the normal dimensions. The cells become completely cornified forming horny masses. The granular layer is in places slightly increased and in others 5 times thicker than normal. The stratum lucidum is clearly outlined. No infiltration of the rete was observable. In places the papillae were flattened and in others hypertrophied. Slight oedema in the papillary and subpapillary layers was observed. The slight infiltration composed mostly of plasma cells and to a less extent of round cells was limited to the papillary layer and located in the vicinity of the blood vessels.

**Differentiation**—Fox (1940) who has discussed the report of Saenz and his associates has pointed out that the hyperkeratotic lesions of the palms in pinta were new to him and that the positive serologic reactions of the spinal fluid had not been observed in other countries. Also the occurrence of juxta articular nodules had not been cited before.

Saenz points out that in regard to differentiation from syphilis two points seem to be of importance. First pinta is limited almost entirely to the colored race. At least he has observed only 2 cases in white persons and he states that in Mexico as well as in Colombia all the patients have been Indians. He thinks it would be an unusual type of syphilis to be limited to the tropical belt of America and as pinta has never been reported in the United States or Europe or in the tropics of Asia and Africa this would be enough to rule out syphilis.

The views of Leon y Blanco as to the differentiation of pinta from syphilis and yaws through the experimental inoculation of pinta with the production of a characteristic lesion have already been referred to and the fact that he emphasizes that syphilis does not render the patient immune to pinta.

In view of these recent investigations in regard to Pinta as Stitt has emphasized it seems advisable to designate syphilis, yaws and pinta as forms of treponematoses.

Obviously the mere finding of a spirochaete or treponema in superficial lesions in the skin would not be evidence that such an organism is the etiological factor. However from the reported experimental inoculations and the repeated transmission of the disease in human beings from cases of pinta to healthy individuals and with the treponema always present in the lesions so produced the etiological importance of the treponema would seem to be demonstrated.

**Treatment**—The treatment of pinta is the same as described for yaws. See p 423. Pardo Castello and Ferrer (1942) have found that Mapharsen has proved highly effective. The superficial lesions of the skin quickly yield to treatment. However it has been observed for years that the white atrophic vitiliginous spots do not disappear under treatment. In some of the cases the Wassermann reaction after the disappearance of the lesions

and the sanitary conditions each separate region requires detailed study. However it may be observed from it that on the whole dysentery is more prevalent in warm countries than in temperate and cold areas.

### PROTOZOAL DYSENTERIES

Of the *protozoal* forms of dysentery *amoebic* dysentery produced by *Endamoeba histolytica* is the most important and by far the most common. This disease will be discussed at length in Chap. XIV and *Bacillary Dysentery* in Chap. XVI. Other forms are described in this chapter.

**Balantidiasis, Ciliate Dysentery** *Balantidial Dysentery* — *Balantidial* dysentery due to infection with the ciliated infusorium *Balantidium coli* is a much rarer disease than either *Amoebic* or *Bacillary* dysentery. It has no special geographical distribution. Cases may be encountered in many parts of the world and a number have been reported in the United States more recently in Texas and North and South Carolina. Young (1939) reported 7 cases in the latter state among 142 insane hospital patients. It also has been frequently observed in Central and South American (Venezuela \* Brazil and Argentina) and in the Philippine Islands, India, China, Indo China, Siam, North Africa, Egypt and the Sudan.

Many cases have been reported from northern and southern Europe. Dopter (1924) enumerated 232 cases among whom were 143 Europeans. In northern Europe cases have sometimes occurred among individuals who prepared sausages. The parasite is common in the pig which is a natural host and in such instances infection has occurred by the transference of the parasite usually in the encysted stage from the hands to the mouth while handling the intestine of infected pigs. Infection also may occur from eating raw sausage. Craig and Faust (1937) point out that considerably over 25 per cent of the recorded cases give a history of direct contact with pigs while the use of the excrement of pigs as fertilizer may be the source of some infections as the cysts are quite resistant to physical and chemical agencies.

It seems probable that this parasite was first seen by Leeuwenhoek (1675) in the discovery of the protozoa in the examination of his own excreta after a painful diarrhoea. Dobell however suggested that it may have been another parasite perhaps *Giardia lamblia* (*Megastoma entericum*) that Leeuwenhoek saw but without giving any proof of the suggestion. The parasite was first definitely described by Malsten in 1857 in a case of ulcerative dysentery and by Leuckart in swine in 1861 under the name of *Paramaecium coli*. Stein especially described the parasite in 1863 and transferred it to the Genus *Balantidium*.

### CLASSIFICATION

**Ciliata (Infusoria)** — The parasite is classified in the class Ciliata in which the organisms move by means of cilia. The Infusoria are the most highly developed of the Protozoa.

The bodies of Infusoria are oval and may be free or attached to a stalk like contractile pedicle as with *Volvoxella* or they may be sessile. The cilia which are characteristic may be markedly developed around the cytostome (mouth) and serve the

100 cases in a year Faust was told



## Chapter VIII DYSENTERY

### *Protozoal and Helminthic Infections*

The term dysentery included in the older literature a group of diseases in which intestinal discharges containing blood and mucus were a common symptom but which differed more or less from a clinical standpoint and had an entirely different etiology. Hippocrates employed the term dysentery to denote the passage of liquid stools while Galen included the presence of mucus and tenesmus as symptoms of dysentery. The term 'dysentery' as used in this respect may be said to be somewhat analogous to that of 'fever' and as we today separate and speak of different forms of fever according to the different symptoms or causative agents so with our present knowledge we have come to recognize a number of different forms of dysentery in all of which at some stage of the disease at least, mucus and bloody discharges from the intestine are a common symptom. These may be classified as (1) *protozoal* (2) *helminthic* or verminous and (3) *bacillary* dysentery.

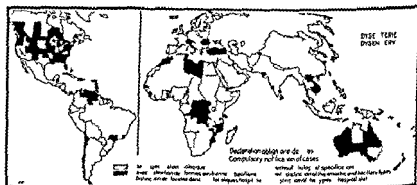


FIG. 110.—Compulsory notification of dysentery cases in the world, 1935 (Epidemiological Intelligence Service of the League of Nations)

In regard to the accompanying map prepared by the League of Nations (Fig. 110) illustrating the geographical distribution it should be noted that comparatively few countries make a distinction in their official statistics based on notifications from physicians, between bacillary and amoebic dysentery. The map of the distribution of dysentery is therefore based mainly on data from clinical and epidemiological publications and from laboratory reports extracted from the medical literature of the whole world. Since there are considerable variations in the incidence of dysenteric affections according to the climate (latitude and altitude)

and the sanitary conditions each separate region requires detailed study. However it may be observed from it that on the whole dysentery is more prevalent in warm countries than in temperate and cold areas.

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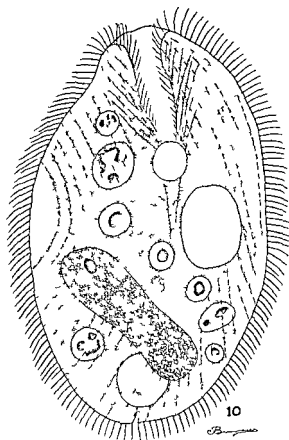


FIG. 111.—*Balantidium coli* from human intestine  $\times$  about 1500 after Wenyon

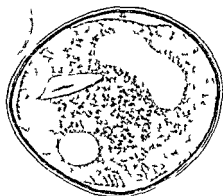


FIG. 112.—*Balantidium coli* cyst as seen in living condition in faeces of pig ( $\times 1000$ ). The elongated macronucleus, two vacuoles and an angular inclusion body are visible. (After Dobell and O'Connor 1921)

purpose of directing food into the interior while others act as locomotor organs. The body is enveloped by a cuticle which may have only one opening or slit to serve as mouth or it may have a second one a cytopyge or anus. Usually the faecal matter is ejected through a pore which may be visible only when in use. They usually have a large nucleus and a small one. Infusoria tend to encyst when conditions are unfavorable (as when water dries up in a pond). When the cilia are evenly distributed over the entire body of the ciliate it is classified in the order Holotricha when ciliated all over but with more prominent cilia surrounding the peristome in the order Heterotricha. It is to this last order that the Infusoria of man belong.

The only species of the class which is pathogenic for man is *Balantidium coli*. It glides along swiftly by movements of its cilia and multiplies by transverse division of the body into two. It also produces resistant cysts. The unencysted ciliate or trophozoite is oval in shape as seen in the faeces and varies considerably in size from about 60-100 $\mu$  in length by 50-70 $\mu$  in breadth. Blacklock gives dimensions as great as 200 $\mu$  by 120 $\mu$ . The larger forms usually occur in pigs. At the anterior end there is a depression or peristome which leads into the mouth or cytostome. There is no definite intestine. At the posterior end a depression is visible named the anal aperture or cytopyge. The body surface is covered by a delicate pellicle which has a striated appearance the striae running obliquely around the body and being formed by the cilia which cover the whole organism. The cilia in the neighborhood of the peristome are distinctly longer than those covering the general body. Beneath the pellicle the clear ectoplasm which surrounds the endoplasm may be observed. Two contractile vacuoles are present a large one situated anteriorly and a much smaller one posteriorly. These vacuoles pulsate at intervals the posterior one apparently emptying into a small tube connected with the surface through the anal opening. Food vacuoles are also usually present. The endoplasm also contains a large kidney-shaped nucleus the macronucleus and a small nucleus the micronucleus in its concavity.

In preparations haemadened and stained with haematoxylin the longitudinal striation in the cilia may be easily observed. The cysts when unstained measure from 45-65 $\mu$  in length have a double outlying cyst wall and are spherical or oval in shape greenish or yellowish in color. When first formed they contain a single *Balantidium*. Sometimes 2 individuals may be observed within a cyst which is an illustration of conjugation and not of multiplication. Encystment appears to be a purely protective process no multiplication occurring within the cysts.

In 1900 the writer studied the infection in the Philippine Islands and gave the first description of the pathological histology showing that the parasites invaded the mucosa muscularis and submucosa and were found in large numbers in the blood vessels of the submucosa and along the intermuscular septa causing round cell infiltration an increase in eosinophile cells accumulations of polymorphonuclear leucocytes later undermining of the mucosa and eventually the production of ulcers. Askanazy later reported similar results. It has also been shown by Bowman and others that the parasite may invade the mesenteric lymphatic gland. The dysentery produced is more commonly of a chronic type and the ulcers are frequently deep and burrowing in the submucosa.

Walker in 1913 was able to produce experimental intestinal lesions in monkeys by injection of *Balantidium coli* from man or other infected monkeys. Infections were obtained either by feeding encysted balantidia or by injecting into the rectum the vegetative motile forms of the parasite.

In the early lesions there is vascular dilatation with minute haemorrhages round cell infiltration and eosinophilia. Later ulcerations and abscesses in the submucosa develop in a number of instances.



Fig. 213—1. *Halantidium colis* in villus of the large intestine. 2. Parasite in the lymphatic gland. (Army Pathological Laboratory, Manila, 1900.)



FIG 114—1. P. t. passing through muscularis mu. of the lrg. intestine  
 a P. t. n. bl. d. v. l. of the submucos. (Army Pathol. Geol. L. b. r. tory  
 Manu. 1900.)

Ratchffe (1934) has reported a study of the lesions in man and in pigs. He has shown that in many cases swine carry the parasite without evidence of disease or invasion of the intestinal wall by the parasite. However, when the swine which harbor balantidia were infected by an organism of the *Salmonella* group, the ciliates invaded the intestinal tissues. He believes the protozoa elaborate a cytotoxic or necrotizing substance that causes tissue degeneration and later ulceration of the mucosa, with the development of undermining ulcers.

The parasite has been found in other animals besides the pig—the chimpanzee and orangoutang—and Harlow Brooks reported an epidemic of dysentery in the orangoutangs in Central Park, New York, due to this parasite.

Studies by Hegner and Nelson (1935) at Johns Hopkins confirm the fact that the species of *Balantidium* of man, swine and chimpanzee are apparently the same. *Balantidium* has also been encountered in the wild pig of the Philippines and in the rat. Nelson (1935) has been able repeatedly to infect rats with the species obtained from the chimpanzee. Awakian (1937) has also pointed out that rats may be naturally infected, show intestinal lesions and be carriers of the human species of *Balantidium*. The parasite in the guinea pig *B. coli* and the monkey *Macacus rhesus* *B. simiae* are apparently different species. Knowles (1934) who fed this species from *Macacus rhesus* monkeys to human volunteers in Calcutta did not succeed in infecting them. Recent observers, Hsiung (1938) and Fusthy (1938) confirm McDonald's contention made in 1922 that there are 2 species of *Balantidium* which infect the pig. These are *B. coli* and *B. suis*, the former being of larger average dimensions than the latter.

The parasite can easily be cultivated in artificial media. In 1921 Barrat and Yarbrough reported the successful cultivation of it from a case in North Carolina in a media containing inactivated human blood serum and 0.5 per cent salt solution—1 part of serum to 16 parts of the salt solution. Partial anaerobic conditions favor the growth. Subsequently several observers have cultivated the parasite successfully in Ringer's serum medium to which a small amount of rice starch is added. It can also be cultured in any of the media satisfactory for the cultivation of *Endamoeba histolytica* and Atchley (1934) found that the addition of filtered aqueous faecal extract in proportions of about 1:4 by volume still further favors the growth of the parasite.

Nelson (1940) has also employed successfully one part intestinal contents diluted with 9 parts of Ringer's solution. The organism has not been cultivated in media free of bacteria.

#### SYMPTOMATOLOGY

The most common symptoms produced are those of chronic dysentery and they are often similar to those observed in amoebic dysentery. There may be colicky pain, a distended and painful abdomen, with a furred tongue and loss of appetite. The number of stools vary with the stage of the disease. Frequently from 8 to 15 are passed during the day.

They may be of the consistence of porridge or fluid alkaline in reaction often contain mucus and sometimes blood corpuscles and leucocytes. The dysentery is often of a very chronic character. Many of the cases show cachexia and anemia. There is no leucocytosis.

Pinto who reported 9 cases from Brazil and Walker in the Philippine Islands point out that some of the cases may be mild and that there may be carriers of the parasite without symptoms. Manlowe has also



FIG. 115.—Chronic ulcerations of the colon. (After Bowman Philippine JI Ser 1909.)

emphasized that there may be intestinal ulcerations and no clinical symptoms. Serra (1931) who found 4 cases in Puerto Rico observed symptoms of dysentery in only one.

### PROGNOSIS

In regard to prognosis of 111 severe cases collected by the writer the mortality was 29 per cent. However the mortality of 57 mild cases was but 7 per cent. Of 132 cases diarrhoea or dysentery was present in 130. In 40 necropsies 36 showed ulceration of the large intestine and chronic catarrh was present in 3. In one case the condition of the large intestine was not noted.



## PROPHYLAXIS AND TREATMENT

**Prophylaxis**—In relation to prophylaxis cleanliness and disinfection of the hands, especially in those who work among pigs such as swine herders butchers, etc is of importance. Also the avoidance of uncooked sausage, and the disinfection of excreta of infected individuals. The protection of food and water from the dejecta of infected animals is of importance and also must be considered. The cysts may remain unchanged in moist faeces for weeks but are quickly killed by drying or by direct sunlight.

**Treatment**—There is no specific treatment and treatment is frequently unsatisfactory. Emetine and ipecacuanha have often been found to be ineffective. However Kipschidse (1928) in the treatment of 22 cases in Tiflis of which 3 died found that emetine injections in large doses 0.05 to 0.06 gm, in 15 to 20 injections gave the best results. Enemata of the organic compounds of silver, such as protargol have sometimes given good results and have apparently caused the eradication of the parasites. Stovarsol and acetarsone have also been recommended. Thymol and oil of chenopodium or arsenical preparations (carbasone) by mouth and yatren by enemata have also been recommended for treatment sometimes with success.

Among recent reports of successful treatment are the following. E. C. Cort treated 17 cases in Siam by enemata of 15 cc of oil of chenopodium in 150 cc of olive oil. All of the patients were freed of the balantidium infection. In one case a second treatment within 24 hours of the first resulted in symptoms of chenopodium poisoning\*. Bank (1935) in one case found emetin  $\frac{3}{4}$  grain daily for 6 days and thymol 16 grains of no benefit. Finally 3 yatren enemas were given 8 oz of a 2.5 per cent solution one on each of 3 consecutive days. These cleared the stools of protozoa and the unfavorable symptoms ceased. McLenzie and Bean (1938) have treated a case by running into the large intestine 2 pints of Loeffler's methylene blue after the colon had previously been washed out. The treatment was repeated the next day. The ciliates were no longer found but gradually diminishing numbers of cysts were passed for a fortnight when the treatment was repeated after which no further ciliates or cysts could be detected. E. Silva in Brazil reported 10 cases in which the usual forms of treatment by yatren emetin etc had proved unavailing. The patients were given a strict milk diet 350 cc 6 times a day. In all but one of the patients the clinical symptoms and the parasite disappeared. In the one exception there was clinical improvement but the protozoan was still found. This patient was treated in addition with parovyl (a proprietary amoebicide essentially the same as acetarsone). The diarrhoea ceased and the balantidia could no longer be found. Bercovitz (1943) says chiniofon has given excellent results. Young (1943) has treated 7 cases successfully with carbarsone.

Several other species of *Ciliata* have been reported in man.

*Nyctotherus faba* was said to have a kidney shaped body and to measure about 25 by 15  $\mu$ . A large contractile vacuole was noted at the

The toxicity of such doses must be emphasized. Diaz (1943) of chenopodium in 25 cc of oil by rectum. The child died several

posterior end and a large nucleus in the center with a small fusiform micronucleus lying close to it. Schaudinn (1889) observed this parasite in the stools of a man in Berlin and it has been reported in a patient in one instance in Italy and in another in Brazil. Nothing is known regarding the life history of this organism, its method of transmission or its relation to disease. These cases may have been examples of accidental invasion.

Schaudinn also reported a small species *Balantidium minus*. It was found in the stools together with *Nyctotherus faba*. Castellani (1905) also found in one case in Africa a species which shows slight variations which he named *Ictotherus africanus*. Wichterman (1939) does not believe that any species of *Ictotherus* is parasitic for man and that all the species found in the stools have been either free living or coprozoic protozoa.

**Dysentery in Malaria and Kala Azar as a Complication.**—Dysentery also sometimes occurs as a complication in at least 2 other important protozoal diseases. First in certain severe pernicious infections with malaria in which the capillaries of the intestinal mucosa are filled with parasites. In fatal cases of malaria with dysenteric symptoms the mucosa of the intestine at autopsy may be congested and dark red in color or have a mottled appearance as in catarrhal dysentery while the contents may be blood stained and contain mucus. The malarial parasites and pigment together with swollen endothelial cells may form veritable thrombi and occlude the vessels. When this occurs in the brain cerebral symptoms of malaria occur and when in the intestine sometimes dysenteric symptoms. In the latter case the capillaries of the mucosa and villi may be filled with parasites, the epithelial cells necrotic. Whether the dysentery owes its origin to the malarial toxine in especially susceptible individuals is not clear. It has been suggested that the epithelial injury predisposes to secondary bacterial infection. The submucosa and deeper layers escape injury unless secondary bacterial invasions occur.

Manson Bahr (1939) who studied malarial dysentery notes the following pathological changes: (1) intense infection of the mucosal vessels with parasitized cells, (2) necrosis of the epithelium, (3) leucocytic infiltration of the tissues subjacent to the necrotic zone, (4) invasion of the necrosed tissues with bacteria.

Craig who has reported a series of cases has also observed similar changes and found ulceration of the mucosa.

Malarial dysentery was studied especially in the World War by Manson Bahr, Dudgeon and Clark and Graham. In a number of cases secondary infections of both bacillary and amoebic dysentery occurred. Both Manson Bahr and Arafah (1930) have described the sigmoidoscopic appearance of malarial dysentery as being characteristic. A diffuse hyperaemia and swelling of the mucosa was observed not unlike the appearance of the early stages of bacillary dysentery. A superficial necrosis of greyish or yellowish white patches on the surface of the gut was also

visible. When these were swabbed or scraped a congested and superficially ulcerated area was revealed. Cases have been reported in which the diagnosis of malaria was first made by finding malarial parasites in the blood cells which were present in the stools. Under anti malarial treatment through the sigmoidoscope, healing of the intestinal lesions was observed.

### TREATMENT

All cases of enteritis in association with subtertian malaria should receive immediate treatment. It is probably best to commence treatment by intra muscular injections of quinine bihydrochloride 10 grains but in more urgent cases it may be necessary to inject the same amount intravenously. Later antimalarial treatment with further doses of either quinine or atevrin should be given. In order to check the diarrhoea bismuth and opium in the form of pulverized ipecac et opii should be employed. If diarrhoea persists in spite of these measures, gentle lavage of the bowel with normal saline or 2 per cent sodium bicarbonate may be advantageous.

**Leishmania Dysentery**—In visceral leishmaniasis or kala azar dysentery may also occur as a complication in advanced stages of the disease. Visceral leishmaniasis is a chronic febrile disease caused by *L. donovani*.



FIG 116—Large intestine kala azar dysentery showing lesions of the mucosa of the large intestine. Leishmania found in films from the intestinal mucosa and in spleen.

discovered by Leishman and Donovan independently in 1903. Its most striking characteristics are the persistent fever, anaemia and cachectical condition with ultimate great enlargement of the spleen and liver. Great emaciation is in marked contrast to the prominent abdomen due to the large spleen and liver. The disease is described in detail in Chap V. In cases which are complicated with dysentery the *Leishmania* are often found in large numbers in the mucous membranes of both the small and

large intestine. Sometimes polypoid masses with necrosis of the epithelium and superficial ulcerations may result. Such cases have been described especially by Christophers, Archibald and Perry. In 2 fatal cases described by Perry the jejunum appeared thickened without ulceration and each villus was changed into a swollen polypoid appearance. The epithelium covering the villi had disappeared. The internal structure of the villi showed an intense proliferation of the endothelial cells lining the lymph channels. *Leishmania* were found in small numbers in the submucous coat in the endothelial cells and in those at the base of the villi. In the center of the villi they were present in the endothelial cells in enormous numbers. Meleny found from the experimental infection of hamsters with *Leishmania* that the intestinal mucosa frequently showed massive accumulations of the parasites but in hamsters the overlying intestinal epithelium was not destroyed.

Shortt and his associates have demonstrated the presence of *Leishmania* in the stools of kala azar dysentery. There was much blood and mucous in the stools and *Leishmania* was demonstrated in the exudate on 2 successive days. Jemma and Christina in cases of infantile kala azar in Sicily observed the presence of enterocolitis associated with circular ulcers in the large intestine. In India in many instances either bacterial dysentery or amoebic dysentery has been found to occur as a terminal complication in kala azar. Rogers noted dysentery as present in 25 per cent of the cases of visceral leishmaniasis. In one series of advanced cases it occurred in 70 per cent.

The treatment of the condition by antimony compounds is described in Chap. V.

### INTESTINAL COCCIDIOSIS

Coccidia are common parasites of the intestine of different animals and *Coccidium* or *Eimeria sturni* is the cause of an acute form of disease known as red dysentery of cattle in which there is usually high fever and the animal becomes greatly emaciated. Theobald Smith and Graybill in 1918 found it in the United States to be the cause of an acute form of dysentery in calves. Henry in 1932 showed that intestinal infection of guinea pigs with coccidia (*Eimeria*) practically always results in diarrhoea and frequently with passage of mucus. Extensive pathological changes were noted in the large intestine. Tyzzer has also made a complete study of the infection in gallinaceous birds with *Eimeria* and described pathological changes in the intestine. The parasites are found especially in the epithelial cells, haemorrhages from the mucosa being common. Dogs and cats are frequently found infected with species of *Isospora* invading the epithelial cells of the small intestine. A closely related if not identical species of *Isospora* has also been found in man associated with intestinal disturbances.

### CLASSIFICATION

The parasites belonging to the order Coccidia are found within the epithelial cells of the intestine and the organs connected with it especially the bile passages. They

grow within the cells which they gradually consume and finally multiply asexually (schizogony) forming numerous minute falciform merozoites. These escape from the disintegrated cell and invade fresh epithelial cells in which the cycle is repeated. Finally certain of the parasites develop into sexual forms (gametocytes). The male (macrogametocyte 20 to 30 $\mu$  long) divides into a large number of minute sperm like bodies provided with two flagella (microgametes) one of which penetrates into and fertilizes the female macrogametocyte. Fertilization is extracellular probably in the lumen of the gastrointestinal tract. The fertilized macrogamete then develops an exceedingly resistant membrane about itself and is known as an oöcyst. The oöcyst is passed in the faeces and constitutes the infective stage of the parasite. Its contents divide into 2 or 4 secondary cysts (sporocysts) within which a varying number of falciform sporozoites develop. When ingested these are liberated, penetrate the epithelial cells and start the asexual stage of development. No intermediate host is required.



FIG. 117—  
1 m 14 1 da  
Oöcyst contain-  
ing four spores  
in each of which  
two sporozoites  
are developing.  
(After Metznér)  
infected meat and

These parasites are common and infect a great many species of vertebrates. Cases of human infection which are rare have been attributed to the two genera *Eimeria* and *Isospora*. In *Eimeria* the oöcysts contain 4 sporocysts, each of which contains two sporozoites. In *Isospora* there are 2 sporocysts, each with 4 sporozoites.

*Eimeria stiedae* (*Coccidium cuniculi* C. ovisforme) is a very common parasite of rabbits invading the epithelial cells of the bile passages and forming small yellowish nodules in the liver. Infection of the liver in man has been reported in about 5 human cases. Craig (1940) gives the name to this species of *Coccidium gubleri* and says the oöcysts 20 $\mu$  in length are considerably smaller than in *E. stiedae*. The oöcysts of *E. stiedae* (in the faeces) are about 40 by 20 $\mu$  oval with a double lined integument. Oöcysts of other species of *E. meria* have been reported in human faeces but according to Magath (1935) these were accidentally swallowed with

**Human Cases of Infection with *Isospora Hominis* (I. Belli)**—Some 225 cases of intestinal infection with coccidia (*Isospora hominis*) have been reported in man since 1915 when the parasite was found in soldiers from the Mediterranean region the first infections having been reported by Woodcock and by Wenyon. Virchow first noted the presence of this parasite in a human being in the intestinal villi the organism being subsequently named *Isospora hominis*. Eimer later found similar bodies in the intestinal epithelium in two autopsies in Berlin. In 1915 Woodcock and Wenyon discovered the cysts in the faeces of soldiers. Connal (1922) accidentally infected himself with the cysts of this coccidium. After an incubation period of 6 days he suffered from diarrhoea and typical cysts were demonstrated in the stools. The faeces were liquid of a brownish yellow color and contained incompletely digested material. Charcot Leyden crystals were numerous but no blood or pus cells were present. The stools contained a large amount of undigested material especially fat. The oöcysts persisted for 36 days after which they vanished following treatment and recovery. The symptoms which were neither prominent nor severe consisted of diarrhoea abdominal discomfort flatulence, loss of weight and a certain degree of lassitude.

The oöcysts are ovoid structures with a smooth colorless doubly refractile wall about 18 by 33 $\mu$  in length and 12 by 16 $\mu$  in breadth. When passed in the faeces the zygote is generally unsegmented though

occasionally segmentation into sporoblasts occurs. Within about 24 hours there appear 2 ovoid sporoblasts about 13 by 9 $\mu$  with doubly refractile walls within the original cyst wall each of which contains eventually 4 vermiform sporozoites about 2 by 6 $\mu$  in size.

In addition to this two spored species 2 other species of four spored coccidia have been reported in man namely *Eimeria clupearum*, *E. wenyoni* and *E. sardinae* (*E. otyspora*). These are parasites of the genitalia (the roe) of herrings and sardines which may be ingested by

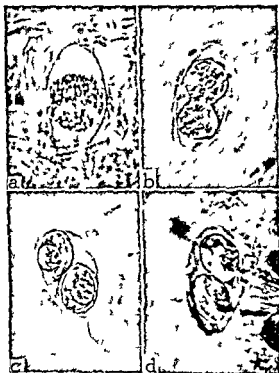


FIG. 18.—*I. o. p. hominis* after Magath. (Am. J. Trop. Med.)

the individual upon eating such fish and passed through the lumen of the intestine unchanged. They were for a time considered to be human parasites.

Wenyon believes that in man as in animals the coccidium (*Isospora hominis*) is a parasite of the intestinal epithelium. Although diarrhoea has been associated with many of these human cases there has been no record of dysentery in man in any case. About 75 per cent of the cases came from the Mediterranean area and near lying countries. They have been scattered through Mesopotamia, Persia, south Russia, Italy, Syria and Turkey. Others have come from north east west and south Africa north

and central China Indo China Bengal, the Dutch East Indies the Philippine Islands Uruguay Brazil Argentina Hawaii and the United States Magath (1935) has made a study of the reported cases One case of infection was observed at the Mayo Clinic This patient apparently contracted the disease in Hawaii Smyly and Kuie (1936) observed a case in which in addition to *Isospora* cysts *E. coli* and *E. nana* were present in the stools

From a study of the literature it would appear that the infection is rare in man Manson Bahr (1939) who also studied the literature of the cases says it is worthy of note that a mild form of diarrhoea with light colored fatty stools consisting to a great extent of undigested material is described by almost everyone who has written on the subject There is also an almost invariable association with numerous large Charcot Leyden crystals

The writer has been able to find the record of only one human case that resulted fatally in which a postmortem examination of the intestine had been made Gaillard (1936) observed in Saigon a case in a child of 2 years of age suffering from acute diarrhoea a pure infection of *Isospora* However the number of cysts found on the day of entrance was very small The eggs of *Ascaris lumbricoides* were also present On the following day it was not possible to find either the oocysts or *Ascaris* eggs On the 4th day the eggs of *Ascaris* were found but still no oocysts The patient died the 5th day At the autopsy no lesions of the intestine were observed nor were any oocysts discovered in scrapings of the mucosa nor in sections Also the author found no *Ascaris* in the digestive tube in spite of the fact that the eggs had been present in the stools It is not clear that any microscopical study of hardened sections of the intestine was made

Manson Bahr (1939) has observed cases of infection in South America in which cysts were present and in which the intestinal symptoms and diarrhoea were regarded as caused by the parasite He concludes from his survey of the literature that *Isospora hominis* is pathogenic for man and probably completes its schizogenic development within the mucous membrane of the intestinal villi the symptoms produced being those of subacute dysentery with the passage of light colored faeces containing much undigested material and an excess of fat its further peculiarity being the large number of Charcot Leyden crystals

**Treatment**—Treatment with large doses of bismuth salicylate together with enemata of 2 per cent sodium bicarbonate has been found satisfactory in the elimination of the cysts and the cure of the condition by Manson Bahr Connal was also cured by the administration of bismuth salicylate and charcoal 3 times daily

#### HELMINTHIC DYSENTERIES

**Schistosomal Dysenteries, Bilharzia Dysentery**—Three species of flukes or trematodes of the genus *Schistosoma* are parasitic in the venous system of man They produce large numbers of eggs which are extruded

through different organs of the body especially the bowel and bladder. Two of these species *Schistosoma mansoni* and *Schistosoma japonicum* give rise to intestinal schistosomiasis and produce dysenteric symptoms while a third species *Schistosoma haematobium* gives rise to haematuria. All 3 of the species however produce pathological lesions in the different organs and especially the liver not only through the toxins secreted by the parasites but by the irritation they themselves and their ova produce.

*Schistosoma mansoni* infection is distributed throughout Africa being most abundant in the Congo basin and in Egypt, French West Africa and Nigeria. In some villages from 40 to 90 per cent of the inhabitants are found to be infected. In the Western Hemisphere it is common in South America, Brazil, Venezuela, Dutch and French Guiana, Puerto Rico and in the Antilles. Jones in 1932 found a quarter of the population of St. Kitts to be infected. The infection was also found to be common in the West African green monkey *Cercopithecus sabaeus* which formerly was imported and has now established itself in St. Kitts. These animals also suffer with schistosomal dysentery.

*Schistosoma japonicum* occurs most commonly in China in the Yangtze valley but it is also found in Southern Japan and Upper Burma and more rarely in the Southern Philippines. It also occurs as a natural infection in cats, pigs, dogs and cattle which animals may serve as reservoirs of human infection.

The life history of the parasite in which species of snails serve as intermediate hosts is described in detail under the general subject of schistosomiasis (Chap. XLVII). In this section merely the subject of schistosomal dysentery will be considered.

Infection is usually acquired by wading or bathing in infected water but may occur through the buccal mucosa from drinking infected water. Penetration of the skin by the cercariae takes place usually giving rise to an intense pruritis and erythema. The parasites 0.1 to 0.2 mm long burrow into a vessel and are carried by the blood stream to the lungs and make their way to the liver and portal veins. About a month after the parasites enter the body there is often a period of fever associated with urticaria, frequently cough, abdominal pain and a leucocytosis with marked eosinophilia. The period required for the development of the characteristic local lesions varies from 6 to 8 weeks to 2 years or more. The lesions differ in detail with the species of parasite concerned. In the case of *S. mansoni* and *S. japonicum* the wall of the colon is particularly involved where most of the eggs are deposited. Many of them however are carried by the portal vein to the liver and in smaller numbers to other organs including the kidneys, lungs and brain. Eventually a fatal portal cirrhosis may be produced. *Schistosoma mansoni* and *japonicum* give rise therefore not only to a chronic dysentery but in many instances to great enlargement of the liver, splenic anaemia and a terminal cirrhosis of the liver.

**Human Pathology**—The pathological changes produced in the intestine are due to some extent to the adult worms but especially to the ova



The worms themselves or the products of their metabolism stimulate local and to some extent systemic cellular proliferation particularly in the liver and spleen. In the interhepatic portal vessels cell proliferation with tubercle formation or local necrosis or infarctions may occur. In the intestinal wall the deposition of the ova leads to marked pathological conditions. Owing to the sharp spines and chitinous cells the ova act as irritating foreign bodies giving rise to proliferation of the epithelial cells and the production of special granulation tissue which contains more or less fibrin. By this proliferation of the epithelium, and the resulting inflammatory exudate the mucous membrane becomes thickened later papillomata are often formed which are frequently very vascular.



FIG 119—Papillomata of the sigmoid flexure and rectum Schistosomal dysentery



FIG 120—Ulcerations of the large intestine Schistosomal dysentery

By rupture of the blood vessels and necrosis of the epithelium ulcerations are gradually formed. When ulcerations of the polypi or papillomata occur in the intestine symptoms of dysentery arise. Secondary malignant changes sometimes develop from the papillomata in the colon and rectum.

Fairley has studied the changes in the tissues in artificially infected monkeys and believes that in these animals the earlier lesions are due to the toxins rather than to mechanical irritation. In monkeys death occurs from the second to the sixth week frequently associated with severe melaena. The earliest lesions occur as pseudo tubercles small whitish nodules which consist of fibroblastic cells with large numbers of eosinophiles. Lampe in Surinam has also observed these pseudo tubercles in the mesenteric glands of human cases of infection. They are also scattered throughout all the organs and particularly on the peritoneal coat of the bowel. They also occur in the mucous membranes where they can

be seen by means of the sigmoidoscope in earlier stages of the infection. In addition to these changes deposits of black pigment derived from haemoglobin are found in the Kupffer cells of the liver. There is often much enlargement of the spleen in the advanced cases giving rise to the condition known as Egyptian splenomegaly.

Manson Bahr (1939) divides the lesions of the colon into 5 types

(1) Those with simple thickening of the mucous membrane and the deposition of eggs in sandy patches in the mucosa

(2) Thickening of the mucous membrane with papilloma formation

(3) Pericolonic tumors associated with papillomata

(4) Polypi of the bowel which may lead to intussusception

(5) Cauliflower excrescences in the neighborhood of the anus

Much diffuse infiltration of the submucosa with the ova gives rise to a catarrhal condition in the bowel wall. Haemorrhages and eventually ulcerations occur which give rise to dysenteric symptoms.

### SYMPTOMS

The general symptoms consist of a high remittent fever with urticaria abdominal pain loss of appetite rigors and sometimes pulmonary symptoms. This stage may last 6 or 8 weeks. After the infection has lasted 2 months or more the symptoms become localized with the passage of dysenteric stools. The symptoms may resemble attacks of amoebic dysentery. Sometimes they simulate those seen in membranous colitis with frequent stools and some tenesmus and in advanced cases the tenesmus is often greater owing to the presence of polypoid growths in the rectum. These may protrude from the anus and resemble haemorrhoids. Perineal fistula and much scarring may also occur.

**Complications**—Frequent complications are ascites and cirrhosis of the liver and also a special form of splenomegaly. Many of these cases result fatally.

### DIAGNOSIS

The diagnosis can only be made with certainty by finding the characteristic ova in the faeces or sometimes in the urine. When the eggs are scanty concentration methods should be employed. Fairley has recommended a complement deviation test for diagnosis. It is based on the discovery that an antigen prepared from an alcoholic extract of the liver of a snail infected with cercariae of *S. mansoni* gives a positive reaction by the Wassermann technique with the blood of patients who harbor any of the three species of Schistosomidae which infect man.

### TREATMENT

Up to a few years ago schistosomiasis was regarded as an incurable disease but in 1915 MacDonagh pointed out the successful use of tartar emetic intravenously in a few cases. However J. B. Christopherson in 1917 made the independent discovery of the value of antimony in the treatment of schistosomiasis and was successful in demonstrating the great

value of this drug. In moderately advanced cases the destruction of both *S. haematobium* and *S. mansoni* may be followed by recovery. In advanced stages of the disease it is often inefficacious. Either potassium or sodium antimony tartrate may be given, the latter being sometimes less toxic. The drug is given intravenously in 1 per cent or 2 per cent solutions. Initial dose for adults is  $\frac{1}{2}$  grain and the dose is gradually increased to a maximum of 2 (rarely to  $2\frac{1}{2}$ ) grains. The total amount of the salt required to effect a cure is placed by Christopherson at 30 grains in adults, but Day found that 24 grains would suffice in many cases. Cranston (1928) says 20 grains. Faust and Meleney have found 22-30 grains intravenously over a period of 18-20 days usually curative. Many prefer to make the injections on alternate days, or 3 times weekly.

### PROPHYLAXIS

The question of diagnosis, prophylaxis and further treatment with organic antimony compounds is discussed in detail in Chapter XLVII under the heading *Schistosomiasis*.

### Other Helminthic Infections

Dysenteric symptoms have also been described from infection with *Oesophagostomum brumpti* and *Gastrodiscus hominis* see Chap. XLV.

## FLAGELLATE DIARRHOEA AND INTESTINAL FLAGELLATES

### Classification, Occurrence and Clinical Manifestations

The flagellates are classified according to their number of flagella and the presence or absence of an undulating membrane and of a blepharoplast. They are adapted to a life in a fluid medium and appear in the stools when the contents become fluid or semifluid. In normal stools encysted flagellates only (of some species) are found. They require only a single host, man, and the human species are probably rarely found in other animals, although a few exceptions are noted below. A number of them can be cultivated on media suitable for amoebae, but there is one notable exception—*Giardia lamblia*.

They can best be detected in fresh preparations in which their active motility makes them conspicuous objects. In a dying condition they may show some pseudo amoeboid movements. To distinguish the flagella it is best to mount them in Gram's iodine solution. For this purpose a clean slide should be used and a vaseline line should be made across it about one inch from the end. A drop of iodine solution is placed on the slide about half an inch from the vaseline line and a suitable portion of the faeces to be examined is emulsified in it. The edge of a square cover glass is then applied to the vaseline line and allowed to drop on the preparation. By pressure suitable thickness of the fluid can be obtained and there need be no current motion. It is desirable to use dark field illumination, as in this way the flagella are distinctly brought out. The India ink method is also applicable. Films may be stained with iron haematoxylin or by Giemsa's method after fixation in methyl alcohol or Schaudinn's solution.

In addition to the species described below, several others have been reported (*Cercomonas Bodo* etc.) as occurring rarely in human faeces, but it is probable that they are accidental contaminations (coprozoic flagellates) and of no practical importance.

Genus *Trichomonas* Donne 1837 This genus includes flagellates having from 3-5 anterior flagella an axostyle an undulating membrane a posterior flagellum and a definite cytostome Three species have been described in man under the names of *Trichomonas hominis* *T. buccalis* and *T. vaginalis* Morphologically they appear to be identical \*

*Trichomonas hominis* (Davaud 186) *Cercomonas hominis* (Davaud 1860) *T. intestinalis* (Leuckart 879) is a very common parasite in diarrhoeal stools see Plate IV Fig 28 p 490 Its distribution is world wide but it is more commonly encountered in man in the tropics and subtropics than in temperate climates It is pear shaped and commonly about 9 by 14 $\mu$  There are 3 to 5 usually 4 flagella projecting anteriorly while another one forms the border of the undulating membrane and projects posteriorly An axostyle passes from the anterior to the posterior end A cytostome is present near the nucleus at the anterior end Multiplication takes place by binary longitudinal division Cysts have not been found The flagellate moves with an irregular circular or spinning motion The organism is usually more numerous in the large intestine and ileum but may inhabit any portion of the small or large intestine It withstands gastric juice and passes through the stomach unchanged

Flagellates identical in morphology have been found in monkeys and Dobell (1934) believes the species he observed in these animals is identical with *T. hominis*

The method of transmission is apparently by contamination of water or food with the vegetative or trophozoite forms as cysts are unknown The incidence of infection varies considerably being higher where the sanitary conditions are poor and hence more common in warm countries Kofoid in the United States examined 2400 soldiers invalided from France and found only 3 or 0 per cent infected Of 34 students he found 0.58 per cent infected Faust in Virginia found 11 or 2.4 per cent of 460 infected At the ambulatory clinic for patients in New Orleans in 4270 examinations he found 0.09 per cent infected On the other hand Fletcher and Jepps in 1934 examinations of natives in the Federated Malay States found 19 or 11.9 per cent infected

*Trichomonas buccalis* (Godey 1917) (Kofoid 1920) —Although some observers have described differences in morphology between this species and *T. hominis* and *T. vaginalis* apparently such differences have related to individual organisms or variations in the organisms due to methods of preparation or of environment Lynch Wenyon Dobell (1934) Craig (1937) believe that all 3 *T. hominis* *T. buccalis* and *T. vaginalis* are identical morphologically *T. buccalis* is found in the human mouth especially in tartar and the teeth and in pus pockets in pyorrhoeal colitis Craig described it in necrotic areas in Vincent's angina and Wenyon observed it in pus in the follicles of the tonsils It has also been found in sputum gingivitis of the lungs and the stomach contents in carcinoma Hezner and Chu also found flagellates which they regard as identical from scrapings at the base of the teeth in monkeys in the Philippines It is a common parasite in the mouth of man and Hogue found it present in 9 or 8 per cent of 5 individuals examined Henshaw (1926) examined 50 inmates of a prison and found 60 or 24 per cent infected Twenty seven per cent of the prisoners were infected but only 4.68 per cent of the prisoners on personnel Beatman (1933) examined 50 patients in a dental clinic and found 49 or 98 per cent infected but 19 normal mouths he was unable to find a single infection Nevertheless others have found it in apparently normal individuals There is no evidence that it is a pathogenic organism though it is much more frequently found in association with diseased conditions in the teeth and gums

While its method of transmission is unknown it is presumed that it may be contracted from infected water or food and also transmitted by kissing and by droplet infection

*Trichomonas vaginalis* (Donné 1837) was described by Donné as the type species of the genus Some of the undivided organisms are larger (12-25 $\mu$  long) than is commonly seen in *T. hominis* and in others the fourth flagellum does not project beyond the undulating membrane Kessel (194) believes it is biologically distinct from *T. hominis* and that the latter will not multiply in the same environment as the former

D. H. Wenrich (1944) describes slight morphological differences Craig (1943) says no differences generally

It occurs in the vagina and in the vaginal secretions when they are abnormal. Hegner and Chu (1930) have found an apparently identical species in the vagina of monkeys. It has also been found in the urine in both men and women and in the former in the prostate.

In the examination of 32 000 prostatic secretions made at the Mayo Clinic, Stuhler (1933) found it in 16 cases. It has also frequently been observed in cases of urethritis and in the bladder. The incidence of infection in the vagina may be high. Kunsler, at the gynecological clinic at Bordeaux, found it present in almost every case but not in healthy women who came to the clinic for accouchement. Brumpt (1933) found it in 8-10 per cent of women examined in Paris and Hegner found it in 16 cases of 32 examined.

Gynecologists very generally regard a form of vaginitis as being caused by it although there has been no demonstration that it produces definite lesions. Dobell reported the infection of the vagina of monkeys with the parasite obtained from women but without the production of lesions. However such an experiment in itself would not be conclusive in a new host in which the environmental conditions would not be the same.

Whether it may aggravate an already existing inflammatory condition of the vaginal tract has not been definitely decided. Craig (1940) points out that the fact that it is generally associated with a form of vaginitis and disappears when the condition is cured is not a proof of its causal relationship, since bacteria and spirochaetes might be present and give rise to the condition as well as the trichomonas any one of which might be removed by treatment.

However, the presence of this organism in the vagina is associated with a white frothy secretion which is usually abundant. The vulva is generally reddened and chafed and the mucous membrane of the vagina and cervix congested. Often there is a deep red mottling. Some observers have suggested that the parasite finds a suitable environment under these conditions rather than that it is the primary cause of the pathological condition. However Kessel (1934) has reported that while cultures of the trichomonas, with the accompanying bacteria would bring about the pathological condition inoculation with the bacteria alone would not accomplish this.

Karnaky (1938) believes that the presence of this parasite is associated with a lowered acidity of the vagina along with a thinning of the epithelium and less glycogen in the cells. He points out that the normal high acidity of the mature human vagina is due to the presence of a rich growth of Doderlein's bacillus (probably identical with *Bacillus acidophilus*). The bacteria are nourished by the glycogen in the vaginal epithelial cells and produce considerable amounts of lactic acid. He believes that trichomonas will not thrive in the normal vagina and that pathological conditions lower the acidity decrease the thickness of the epithelium and reduce the store of glycogen. (For treatment of this affection see p. 471.)

Genus *Chilomastix* Alexeieff (1913) This genus was established to include a flagellate found in the tadpole. The organisms possess 3 anterior flagella, a large cytostome and a posterior flagellum. The cysts are lemon or pear shaped and have a single nucleus, no reproduction occurring in the cyst.

*Chilomastix mesnili* Wenyon (1910) is a common flagellate which differs from the trichomonas in not having an undulating membrane or an axostyle. It has a trophozoite and cystic stage. It is about 6 by 14 (to 20)  $\mu$  in size. The 3 anteriorly projecting flagella are long and slender. See Plate IV Figs 26-27. There is a long prominent slit like cytostome within which there is a short flagellum. The posterior end is very much attenuated and tail like. It moves in a slow deliberate manner with slow rotation of its body. Cysts are about 8  $\mu$  in length ovoid with a small projection at the narrower end and contain one nucleus. Like *T. hominis* it occurs in the large intestine especially in the caecum. However the exact habitat of this flagellate is disputed. Wenyon found it within the lumen of the glands in the large intestine and many regard the large intestine as its most common habitat. Nevertheless some authorities have suggested that it lives normally in the small intestine. The trophozoites are found in fluid or semifluid stools and the cysts in formed or semiformed stools. The trophozoite reproduces by longitudinal division with preliminary fission of the nucleus. No division occurs within the cyst, one organism emerging at the time of excystation.

Transmission from man to man occurs as in *T. hominis*. Its distribution appears to be cosmopolitan. Apparently it was seen by Davaine in 1854 in Paris in cases of cholera. It is difficult to indicate its frequency in man since it has often been confounded with or included in statistics with reference to *Trichomonas*. In the United States Kofoid, Meleney and Bishop and Leathers in different surveys found about 3 per cent of those examined infected while Scott in Jamaica found it in 11.74 per cent of apparently healthy individuals.

This organism is very frequently encountered in cases of amoebic dysentery. It is also commonly found in large numbers in patients suffering from diarrhoeal conditions. Wenyon has observed it in sections of the large intestine and in the intestinal glands. There is however no evidence that it produces lesions in man. Brumpt points out that it is frequently found when there is absence or insufficiency of acidity of the gastric juice.

Genus *Giardia* Kunstler 1882. The genus was established in connection with the flagellate *Giardia* found in the tadpole. The organism is characterized by a bilateral symmetrical pear shaped body containing 2 nuclei. The dorsal surface is convex while the ventral surface is flattened and presents a well defined sucking disk. Eight flagella are present, 4 arise from the middle of the body and 4 from the posterior end. *Giardia lamblia* is the species invading man.

Synonyms *Megastoma enterica* Grassi 1881 *Lamblia intestinalis* Lambl Blanchard 1888

*Giardia lamblia* Lambl (1859) is the commonest flagellate of man. Plate IV Figs 24-25. It lives in the upper part of the small intestine and hence its habits differ considerably from other protozoa that reside in the large bowel. Its distribution while world wide is more common in the tropics. Even in temperate climates as in the United States it is more common in the south than in the north. The parasite is very commonly found in the United States in children under 10 years of age.

Stiles in Washington found it in 13 per cent of boys and 8 per cent of girls.  
Kofoid in 2300 soldiers who served abroad in 5.7 per cent and in 576 men in home  
see 22 per cent.

Wenyon and O'Connor in Egypt 4.1-16 per cent

Jepp in British soldiers in 13.2 per cent

Matthews and Smith in convalescent soldiers in 16.4 per cent

Meleney Bishop and Leathers in Tenn. 14.7 per cent

Faust and Headlee in dispensary clinic patients in Louisiana 16.6 per cent

The flagellate is pear shaped about 10 by 15  $\mu$  in size. There is a deep depression on the ventral surface at the blunt anterior end which enables the organism to attach itself to the summit of an epithelial cell. Around the depression are 3 pair of flagella which are in constant motion. Another pair of flagella project from either side of the blunt little tail like projection. When stained they show a pair of chromatin staining areas at the anterior end. There are 2 axostyles but no undulating membrane. When in motion they have a slow tumbling sort of progression. The cysts are oval 8 by 14  $\mu$  and show 2 axostyles and 4 small nuclei. Reproduction takes place by longitudinal binary fission. Usually an ovoid forms around the anterior end of the body and expanding backwards gradually encloses the tail which is finally retracted within the cyst wall. In a recently encysted giardia sometimes both flagella of the extremity may be seen moving within the cyst. The parasite develops in the cyst so that eventually 2 separate ones and 2 sets of nuclei and accompanying structures lie inside. To the unaided eye it might resemble a four nucleated cyst. These cysts may persist in the stools for years. Manson Bahr reports one case in which they persisted continuously for 10 years.

In one species Hegner (1927) has observed excystation and reports that the division of the body of the flagellate occurs after excystation is complete.

As the organism inhabits the small intestine of man the trophozoites are most numerous usually in the duodenum. The cysts occur more commonly in the lower portion of the ileum in the large intestine and in formed stools. By means of its sucking disk it may attach itself to the mucous membrane.

Hegner, Wenyon, Kessel and Hegner and Chu have found an apparently identical species in monkeys. Rats, mice, guinea pigs, rabbits and dogs harbor flagellates belonging to this genus but it is not clear that they are identical with *G. lamblia*. Simon and Hegner believe that the organisms found in rats and mice are a distinct species though some have claimed to infect rats, mice and kittens with the human parasite. Faust (reported by Craig 1937) has found that *G. lamblia* is readily transmitted to dogs.

The transmission of *G. lamblia* as in *T. hominis* is dependent upon the ingestion of food or drink contaminated by faeces though in this instance it is the cysts that are ingested. These cysts are quite resistant, living in potable water for at least 4 days (Brumpt 1921) and resisting a 0.5 per cent chlorinated water for 2 to 3 days. Wenyon and O'Connor, Braubaud and Root have demonstrated that the cysts of this organism may remain alive in the intestine of flies for 24 hours and that food and drink may be contaminated by fly droppings. It has been suggested that possibly animals might transmit the infection.

**Cultivation**—In spite of numerous trials that have been made the successful cultivation of this organism has apparently not yet been achieved though the organism will live for a considerable time in normal saline solution in which some multiplication may occur.

*Embodomonas intestinalis* (Wenyon and O'Connor 1917) is about 6  $\mu$  long. It is actively motile and pear shaped. Two flagella arise from blepharoplasts on the nuclear membrane of the single nucleus which is near the blunt end of the parasite. The cysts are about 5  $\mu$  long and pointed at one end.

*Enteromonas hominis* (Fonseca 1915) is a minute actively motile pear shaped flagellate with the posterior end pointed. Three flagella project anteriorly and one posteriorly. The small cysts resemble fungus spores. Both of these species are rare and of no clinical significance.

## CLINICAL MANIFESTATIONS

There has been much discussion as to whether any of the three most common intestinal flagellates *Trichomonas hominis*, *Giardia lamblia* and *Chilomastix mesnili* actually play a role in causing either diarrhoea or dysentery. Today the view is generally held that *Trichomonas* and *Chilomastix* are more or less harmless commensals which on the occur

rence of intestinal disturbances from other causes find in the intestine suitable culture media and multiply prodigiously. Their astonishing abundance in stools at such times may impress on the observer their importance. However these flagellates are frequently observed in the stools of perfectly well people and appear never to have given many of them any trouble. Variations in the reaction of the gastric juice (anacidity) or of the intestinal contents seem to favor multiplication but just what role they may play in intestinal diseases is not entirely clear. When present in very large numbers in association with diarrhoea of unexplained origin it is sometimes difficult to decide whether their presence is due to the diarrhoea or whether they actually play a causal role in its production.

In the opinion of Dobell there is insufficient evidence for regarding any of the intestinal flagellates as pathogenic and he believes that parasites of the alimentary canal which do not attack the tissues of the host as is the case with intestinal flagellates are not harmful. He notes that intestinal flagellates are adapted to a life in a liquid medium and appear in the stools when the intestinal contents become fluid or semi-fluid. In normal stools encysted flagellates alone are found. He also points out that no method of treatment has yet been discovered which will remove such an infestation.

These organisms obviously thrive when the contents of the intestine have been rendered abnormal as is the case in different forms of diarrhoea or dysentery. When in the case of chronic diarrhoea very large numbers of these actively motile organisms are present and no other cause for the diarrhoea can be discovered one must at least consider the possibility of their unfavorable action on the host. Manson Bahr points out one cogent point in favor of those who hold that flagellates (*Chilomastix*) can cause a diarrhoea *sui generis*—is that after appropriate treatment such as colonic lavage the active forms disappear from the faeces when the active symptoms subside and when the faeces again become formed the cysts of the parasites appear.

An important point is that the presence of flagellates in the intestines and stools is indicative of faecal contamination of water or food taken by the mother and one must consider the possibility of other pathogenic organisms both bacteria and protozoa having been introduced in this way. The presence of flagellates particularly suggests the possibility of infection with *Entamoeba* and such infection is not infrequently found to be coexistent.

In cases of amoebic dysentery the diarrhoeal attacks which at times occur are often associated with an abundance of flagellates and it has been suggested that the flagellates may be the cause of or increase the diarrhoea.

*Giardia* is an inhabitant of the small intestine and its role will be discussed below p. 469. *Trichomonas* and *Chilomastix* as noted occur especially in the large intestine and are often abundant in the region of the caecum. As they occur so commonly in association with diarrhoeal conditions the term flagellate diarrhoea has come to be frequently applied to them. While as pointed out the pathogenicity of the flagellates is still in dispute conclusive evidence of the production of pathological lesions in the intestinal tract has not been produced by *Trichomonas hominis*. In fact tissue invasiveness does not appear to be an attribute of the genus *Trichomo-*

Wenyon has examined sections of the intestine from 5 fatal cases infected with *Trichomonas*. In one of these the flagellates were found in the lumen of the glands and actually invaded the glandular cells and were distributed throughout the connective tissue. Kessel claims to have infected kittens with this organism and observed superficial necrosis of the mucous membrane. Kessel has also reported its presence in liver abscess pus in conjunction with *Entamoeba histolytica*. Pentimalli reported its presence in the blood of one patient. However further



observations of this nature have not been made and Wenyon has suggested that in his case, the organisms may have reached the interglandular connective tissue post mortem

*Trichomonas vaginalis* has been reported sometimes to cause acute vaginitis with profuse leucorrhoeal discharge. Its significance has been more fully discussed under the description and occurrence of this organism (p 464). No account of histopathologic lesions caused by *T vaginalis* is known.

One of the most striking examples of the pathogenicity of *Trichomonas* in animals is that of *Trichomonas foetus* in heifers as reported by Mazzanti (1900) the parasite being named by Riedmüller (1928). This organism occurs in the ejacula of bulls and natural transmission of the parasite occurs from the infected bull to the heifer. The vagina is thus infected and later the uterus is invaded and pyometra, abortion and sterility may result. The investigations of Riedmüller (1933) and Witt (1934), Andrews and Miller (1938) seem to have established these facts.

Caughen, Callender and Simmons (1937) have described the infection of sparrows and doves with *Trichomonas columbae*. In doves the lesions were found in the throat, crop and region of the oesophagus. The earliest lesions observed by Callender and Simmons in artificially infected birds were white raised macules 1 to 2 mm in diameter. These coalesced and gradually turned yellowish and developed crater like ulcerations. From extensive studies they demonstrated that *T columbae* appears to be definitely pathogenic and that it can injure columnar epithelial cells in susceptible species by contact or possibly by penetration between cells to the basement membrane. It also can make its way along such passages as ducts, bronchi etc. carrying bacteria which either alone or in combination with the *Trichomonas* produce inflammation and abscess formation. There is no evidence that the *Trichomonas* injures squamous epithelium primarily and no evidence that the species actually invade tissues.

Hess (1938) in Egypt has reported the inoculation of 6 females with *Trichomonas genitalis bovis* and that a *T vaginalis* infection resulted in them. In one case of oral administration of massive doses of cultures of *T genitalis bovis* to an inoperable case of cancer there was reported the appearance of the human intestinal trichomonas in the blood and intestine and *T vaginalis* in the vagina. Further experimental investigations obviously are necessary before any conclusions can be drawn from this report.

The careful consideration of the subject of the infections in man compels one to conclude that there is not definite information from which to conclude that these organisms are capable of primarily initiating any pathological lesions. In severe human infections with *Trichomonas* or *Chilomastix* in association with diarrhoea, in some instances their presence seems to give indication of an abnormal condition of the mucous membrane of the intestine. Evidence of the production of an abnormal amount of mucus by the lining cells which favors multiplication of the parasites in large numbers may sometimes be seen in the examination of the stools. Since there is no evidence in the human infection that the flagellates are able to produce lesions, any erosions or ulcerations of the mucous membrane which exist are probably due to bacteria or other protozoa. However a severe infection with flagellates engrafted upon such pathologic conditions may act as an irritating agent and perhaps cause an additional extension of bacteria in the lesions and tend to maintain or aggravate an already existing condition. In this it appears probable that the flagellates may play a secondary role.

## GIARDIASIS

Fantham and Porter called attention to this condition by their report of 187 cases of intestinal giardiasis (lambliasis). The parasite has also been reported both from the gall bladder and the appendix. In giardiasis an acute onset has been rarely reported. Usually 5-6 stools a day with remissions and exacerbations of the diarrhoea have been observed. The stools were generally abundant, often with the consistency of thin dough, of an offensive odor and containing a great deal of mucus. There has been very little colic and no tenesmus as would be expected in the case of an organism invading only the small intestine.

In recent years there has been a decided tendency among clinicians to regard *Lamblia* as pathogenic for man and some have even gone so far as to refer to giardial or lamblial dysentery. Also it has been suggested that it might be concerned with and indeed was the cause of some cases of cholecystitis.

The parasite has been frequently found in duodenal juice obtained by intubation by many clinicians. However there appears to be no convincing evidence that it is connected with disease of the biliary apparatus.

Deschiens (1930) and Brumpt (1936) have found that in the case of the species of *Giardia* which infects mice there is no invasion of the biliary ducts by the organism.

Farse and Jacquot have reported that *Giardia* can penetrate the intestinal mucosa of man and live in the submucosa. An allied species has been found in cysts of the liver of the rat and Basile who inoculated these forms into the peritoneum of the rat produced an infection of the liver and of the mesenteric lymphatic glands. Deschiens had earlier reported that he succeeded in producing serous diarrhoea or dysentery in young cats by introducing per rectum active stages of *G. lamblia*. However there has been no recent confirmation of such studies. Brumpt points out that when present in enormous numbers upon the surface of the intestinal epithelium the parasites may prevent proper activity and proper function of the cells.

Manson Bahr (1939) has reviewed especially the clinical evidence of the pathogenesis of the parasite. (1) It is found in its active state in the largest numbers when the stools are liquid and diarrhoeaic. (2) In the early stages of infection symptoms of gastro-enteritis are present while in the chronic stages the stools contain large numbers of the cysts.

Heubner who has reported 173 cases believes that as a rule the parasite multiplies to such an extent in the small intestine that sooner or later it must give rise to symptoms. First it colonizes in the duodenum and may lie upon the mucous membrane so closely that disturbance of intestinal function is produced.

During the past decade numerous clinicians have also found these flagellates in individuals with disturbances of the gall bladder. A number of these cases have been operated upon. However in many of them even though the parasites have been found in the duodenal juice they were not encountered in the gall bladder at the time of the operation. Thus in ten cases studied by Morenus and Deschiens no flagellates could be found in any case in the gall bladder however in a few cases they have been present.

Spangenberg (1939) has reported upon a patient with symptoms of cholecystitis who had large numbers of *Lambdia* in the liquid withdrawn by duodenal tubing. The gall bladder was removed by operation and the patient made a good recovery. Careful examination of the contents of the gall bladder and the wall of the serial sections failed to reveal any flagellates which however could still be obtained in abundance by tubage of the duodenum. It seems evident that the presence of *lamblia* in fluid withdrawn from the duodenum even in cases suffering from symptoms referable to the gall bladder is not an indication that they occur in this organ.

Bonanno (1939) has observed a series of 37 cases of *Giardia* infection. In 6 of the cases including 3 in which gall stones were visible cholecystectomy was performed. In the bile of the gall bladder of 4 two of whom were cases with calculi flakes of mucus were seen containing large numbers of *Giardia*. All 6 of these cases had shown abundant flagellates by duodenal tubage. Three of the operated cases were followed for a year. Symptoms of colic previously presented had completely disappeared but duodenal tubage still revealed *Giardia* in two. He thinks the flagellates in the bile ducts merely act as mechanical obstructions to the flow of bile giving rise to symptoms of biliary colic though they may irritate to some extent the mucosal lining.

Brumpt (1936) believes that the parasite often produces an entero-colitis of which it is the only etiologic agent and that the presence of thousands of the motile or agglutinating parasites upon the debris of epithelium in the small intestine in cases of mucobilious diarrhoea is convincing of their pathogenic role. He further points out that it is frequent for animals to succumb (as rabbits cats dogs and mice) in the course of experimental infections.

**Clinical Observations**—The usual symptoms have already been referred to.

Veghelyi (1938) observed 155 cases of infection in children between the ages of 2 and 17. Of these 144 were precisely examined and the symptoms compared with those of healthy children of the same age. Thirty-two of the infected gave evidence of various other diseases and 20 gave a positive reaction to tuberculosis. Ninety-two children remained. Some of the infested children were symptomless and some had symptoms of no significance but gastro intestinal complaints anaemia and inadequate development were present in a high percentage of cases. Many had cramp like attacks. Mucus and blood were seen in the faeces in one fourth and two thirds complained of irregular bowel movements. No pathologic condition could be revealed by the physical examination. Of the 92 infested children only 13 attained or surpassed the average weight while 79 were below it. All the symptoms could be explained by the impeded resorption capacity of the intestinal tract. The symptoms disappeared in 29 of 32 children after treatment with acetarsone. Regeneration of the blood of the anaemic children and rapid development of those retarded in weight quickly started after successful treatment. In 3 cases in which treatment was not successful the symptoms were further aggravated. He believed that he had found evidence of mechanical interference with absorption particularly of fats from the intestine, by the layer of parasites adherent to its wall and it has been suggested that this might lead to vitamin deficiencies particularly of the fat soluble one.

Manson Bahr has recently made a special study of 26 cases 24 in men and 2 in women. The chief complaint was of initial diarrhoea of enteric character followed by a more or less chronic condition of intes-

tinal disturbance. Some flatulence was almost invariably present. In general the stools of giardiasis number from 2 to 8 per day. There are usually remissions and acute exacerbations of the diarrhoea, sometimes with passage of considerable bile stained mucus.

Brumpt reports rarely a dysenteric syndrome which may resemble amoebic dysentery but is not ameliorated by emetine. In such instances there may be from 20 to 24 mucus and bloody evacuations per day together with colic and fever. In other instances the stools are abundant, the consistency of thin dough and with an offensive odor and containing a great deal of mucus. Usually there is very little colic and no tenesmus. The abdominal pain varies in intensity. Sometimes there is merely discomfort. In still other cases the dejecta have been described as pale or bright yellow and more offensive than sprue stool which they may somewhat resemble.

In 19 of Manson Bahr's cases the cysts of *Giardia* were present in large numbers and in 2 of these *Entamoeba histolytica* cysts were present as well. In 4 of the cases both free and encysted forms of *Giardia* were present at the same time. Eight of the cases had a previous history of amoebic dysentery and 5 a history of bacillary dysentery while 3 had previously suffered from typhoid and two from chronic indigestion. He examined all cases by means of the sigmoidoscope but there were no appearances in the large bowel which could be described as characteristic.

DeMuro (1939) Stockholm from his studies made on 45 cases found that the symptoms appeared under 4 different clinical forms but enterocolitis was the form observed most frequently existing in 62 per cent of the cases. The main feature was the abnormality of the stools. As a rule there was no blood. After a long time the general condition became improved. Many patients presented a more or less profound anemia. Chemical tests showed as a rule gastric hypochlorhydria. Enterohepatobiliary syndrome in which there existed duodenitis, cholecystitis and hepatitis was observed in 14 of the 45 cases.

### TREATMENT

The treatment of flagellate infections of the intestine has been very unsatisfactory and this is emphasized by the large number of drugs that have been recommended by different clinicians.

Dobell found no method of treatment he employed would remove such infestation. Low noted the tendency of giardiasis to recur and thought many of the reported cases of cure were only temporary. His experiences in treatment with bismuth salol, thymol and cilyn were not encouraging. Dobell and Low in a study in which the methods were carefully controlled tried a number of drugs to eradicate *Giardia* including emetin, bismuth iodide but their results were inconclusive of their value although good results had previously been reported from the administration of ipecac either alone or with calomel. Dobell and Low also had no success with methylene blue, turpentine or beta naphthol.

Manson Bahr (1939) in the treatment of flagellate diarrhoea says that while there does not appear to be any specific treatment for these infections as such the organism may disappear after vigorous lavage of the intestinal canal by simple irrigation of two

Spangenberg (1939) has reported upon a patient with symptoms of cholecystitis who had large numbers of *Lambia* in the liquid withdrawn by duodenal tubing. The gall bladder was removed by operation and the patient made a good recovery. Careful examination of the contents of the gall bladder and the wall of the serial sections failed to reveal any flagellates which however could still be obtained in abundance by tubage of the duodenum. It seems evident that the presence of *Lambia* in fluid withdrawn from the duodenum even in cases suffering from symptoms referable to the gall bladder is not an indication that they occur in this organ.

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### TREATMENT

The treatment of flagellate infections of the intestine has been unsatisfactory and this is emphasized by the fact that no treatment has been recommended by different clinicians.

Dobell found no method of treatment helpful. He noted the tendency of giardiasis to recur and his attempts at cure were only temporary. His experience with emetine, metronidazole and cetyl was not encouraging. In 1942 he reported that the results were carefully controlled but that the treatment was not successful. Emetin bismuth iodide but has not been used since. The results had previously been reported by other workers. The treatment with calomel. Dobell and Low also have reported no success with o-beta naphthol.

Manson Bahr (1937) in the treatment of giardiasis does not appear to be very successful. He reported that the symptoms may disappear after a course of treatment but the infection may recur.

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As *Giardia* inhabits especially the upper portion of the small intestine it was suggested formerly that the administration of drugs by the duodenal tube might prove a more effective method of treatment but this has not been demonstrated.

During 1939 a number of clinicians in Europe and a few in the United States have reported especially successful results from the oral administration of *atebrin*. Gali Valerio and Morrison and Swaalm and Heilmann are among those who are enthusiastic about its use. Morrison and Swaalm have reported the results in 10 carefully studied and treated cases in which they gave a dosage of  $1\frac{1}{2}$  grains (0.1 gm) of *atebrin* 3 times daily orally for 5 days. After one week a similar dose was again administered if necessary. They report that *atebrin* was a completely successful parasiticide in 9 of the 10 cases. Eradication of the parasite caused complete abolition of the symptoms in some cases, moderate improvement in others and no improvement in certain others. They point out that in those cases in which the *Giardia* is a secondary invader the symptoms persist as long as the fundamental cause persists despite the eradication of the parasites. They found no recurrences of infection in their patients up to 2 years following the use of *atebrin*.

**Treatment of *Trichomonas Vaginitis***—Various methods of treatment have been recommended. Karnaky (1938) has employed (1) capsules containing glucose and lactose to stimulate the growth of Doderlein's bacillus (2) boric acid to create an immediate acidity (3) an iodine compound to kill the parasite and (4) cornstarch as a base. He washes out the vagina first with tincture of green soap followed by a boric acid douche after which one capsule is inserted once or twice a day for 2 or 3 weeks. He believes a higher percentage may be cured by this method than by any other. Douches of diluted vinegar have also been recommended.

Falls and Hibbert (1938) have reported excellent results from the use of streptococcus subacidus vaccine but Karnaky failed to observe any benefit from its use in 100 cases. Others have recommended douches of 1 per cent picric acid and 0.5 per cent lactic acid.

Craig (1940) reports that a treatment which is proving very efficient in eliminating the infection is the insufflation into the vagina of a powder containing 12 parts of stovarsol (acetarsone), 2 parts of salicylic acid and equal parts of kaolin and sodium bicarbonate to make a total of 100 parts. The powder is blown into the vagina after thorough cleansing of it with tincture of green soap diluted with equal parts of warm water treatment to be given twice a week.



per cent sodium bicarbonate or by any of the other reagents used for colonic irrigation in bacillary dysentery as the silver salts. This obviously would not eliminate *Giardia* from the small intestine.

Other clinicians have recommended the newer arsenical preparations Stovarsol or acetarsone (an acetyl derivative of oxyaminophenyl arsenic acid) has been reported in doses of 4 grains daily for 8-10 days by several observers to exert specific action upon *Trichomonas intestinalis*. However others have reported failure with both stovarsol and yatren.

In the United States treparsol (meta amino-para oxyphenylarsenonic acid) in doses of 0.25 gm. 3 times a day for 4 successive days has been advised. After an interval of 8 days the course of treatment is repeated. A third course of treatment is given after another interval of 8 days. Under such a regimen, it has been reported that many of the patients will be freed of their *Giardia* cysts at the end of the third course of treatment. Such treatment has given rise to toxic manifestations caused by the arsenic in from 2-3 per cent of the cases and in such cases treatment must be discontinued immediately.

Craig (1937) points out that emetin, chiniofon, treparsol, acetarsone and carbarsone are usually of little value in the elimination of this infection but they have been reported as being the most useful for treatment. He emphasizes that there is no known specific that will eliminate the infection in all cases.

With reference to *Giardiasis*, Whittingham has tried an intensive form of treatment consisting of large doses of sodium bicarbonate followed by calomel, magnesium sulphate, thymol, emetin, bismuth iodide and high lavage without producing any permanent results. On the other hand, Noir and Deschiens believe that carbonate of bismuth is specific if given in doses of 30 grams daily in the morning on an empty stomach for 8 days and the course repeated after a 2 day interval. Other authors have advocated intravenous injections of salvarsan. Da Silva (1938) has reported the successful treatment of 33 cases by injections of full doses of neosalvarsan followed by the oral administration of stovarsol or in other cases of yatren.

Manson Bahr has adopted the yatren treatment giving yatren pills by mouth and yatren retention enemata  $2\frac{1}{2}$  per cent over a period of 10-12 days. He says that while the treatment has been followed by amelioration of the symptoms there is no evidence to show that the parasite was permanently banished from the bowel.

Hegner in the study of 3 infected rats and 3 human cases showed that a carnivorous diet was unfavorable to the existence within the intestine of flagellates of the genera *Giardia* and *Trichomonas*. DeLangen has also advocated the exclusion of carbohydrates and the substitution of a meat diet for eliminating the intestinal infestation with the flagellate. Manson Bahr (1939) says that he has also successfully alleviated the symptoms by such dietetic treatment.

Brumpt (1937) in experiments on *Lambia* has infected mice and found that quinacrine in 1 per cent solution given orally for 5 days was able to bring about a cure in 80 per cent of the animals to which it was administered.

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doubt upon the question of the pathogenic action of amoebae. In 1880 however Kartulis published the results of his investigations upon over 150 cases of Egyptian amoebic dysentery in which amoebae were found in the stools of every case while they were not found in the dejecta of 30 normal individuals examined for control purposes. Kartulis emphasized the fact that the amoeba was the cause of the tropical dysentery studied. In 1887 he found this parasite in cases of dysenteric abscess of the liver. In 1890 Osler in Baltimore discovered amoebae in the contents of a liver abscess and in the stools of a patient who was suffering with chronic dysentery which he had contracted in Panama. Other cases in which amoebae were found in the stools were then reported in the United States by Musser Stengel and Dock. In 1891 Councilman and Lafleur published a complete study of fifteen cases of amoebic dysentery. They described histological peculiarities by which this form of flux differs from other types and concluded that amoebic dysentery should be regarded etiologically, clinically and anatomically as a distinct disease. It is to their important monograph that we owe much of our present knowledge of the pathology of this disease.

In 1894 Kruse and Pasquale by their extensive studies in Egypt did much towards confirming our belief in the existence of amoebic dysentery as a separate disease with a specific etiology and Harris in 1899 by his investigations also added important data in the differentiation of the malady in America. In 1900 the writer showed that the prevailing dysenteries of the Philippine Islands could be divided into 2 distinct forms, one of which owed its origin to a variety of amoeba (*Amoeba dysenteriae*) and the other to a species of bacterium (*Bacillus dysenteriae*). He also differentiated the pathogenic from the innocuous amoebae of the intestine by experiments on cats producing on the one hand typical amoebic ulcerations in these animals with amoebae from dysenteric cases and on the other showing that *Amoeba coli* and free living amoebae grown in straw infusions were incapable of producing specific infection in such animals (see Fig. 122).

Leonard Rogers in 1903 increased our knowledge of the disease as a separate infection in India where its existence had previously been frequently denied and showed clearly its association in that country with liver abscess.

Schaudinn (1903) confirmed the idea previously demonstrated by Quincke and Roose Kruse and Pasquale (1894) and the writer that a pathogenic and non pathogenic amoeba occurred in the human intestine. However Schaudinn in addition made important zoological studies and gave extended morphological descriptions of the forms of amoebae found in the human intestine.

Since this time many important publications upon the amoebae and their relation to disease in man have appeared. In 1904 Vedder called attention to the amoeba and properties of ipecacuanha and emetin and shortly afterwards Rogers and others applied and extended these results. From these investigations it was demonstrated that emetin constitutes one of the most specific drugs known.

Among other important publications relating to amoebae may be mentioned those of Jurgens (1902-06) Meserve and Clegg (1904) Prowzek (1904-12) Hartmann (1908) Walker and Sellards (1908-13) Craig (1905-1937) Wenyon (1916-17) and DeBell (1919).

A number of these publications have served to emphasize the different species of amoebae which occur in the human intestine and the differentiation and the fact that while some are pathogenic others are harmless commensals of man.

In 1918 Cutler reported the cultivation of *E. histolytica* and the more successful isolation of this organism was demonstrated by Boeck and Drbohlav in 1924.

**Geographical Distribution and General Prevalence**—The disease has a wide geographical distribution. It occurs sporadically in most subtropical and temperate countries but it is much more frequent in tropical ones. In the tropical and subtropical portions of America, Asia and Africa and also in the West Indies, the Malay Archipelago and the Philippine Islands it has usually been the prevailing form of dysentery.

## Chapter XIV

# AMOEBIASIS

### AMOEBIIC DYSENTERY

**Synonyms** — Amoebic colitis amoebic enteritis endamoebiasis entamoebiasis

**Definition** — By the term amoebiasis is understood infection with the pathogenic amoeba *Endamoeba histolytica*. However, the important pathological conditions produced in man by this organism result from primary infection of the intestine. The organism establishes itself in the large intestine penetrating into the tissues of the intestinal wall and often causing a characteristic type of chronic ulcerative colitis, associated (in some cases) with the clinical symptoms of dysentery. From the intestinal lesions, the organisms frequently metastasize through the portal veins to the liver causing a hepatitis or abscess or rarely to other tissues (lungs brain etc.)

Four other distinct species of amoebae are known to establish themselves in the gastro intestinal tract of man, but it is generally believed that they are harmless saprophytes growing in the intestinal contents and they have not been shown to invade the tissues or definitely to cause disease symptoms.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** — Dysentery is a disease of great antiquity, for it is referred to in some of the most ancient writings upon medicine. However, the first step towards the differentiation of a variety of amoebic origin dates from 1859 in which year Lambl called attention to the presence of amoebae in the intestine of a child who died of enteritis. Losch, in 1875 in Russia also found amoebae in the dejecta during life and in the intestinal lesions at autopsy of a case of chronic dysentery. He gave a description of the parasite which he observed naming it *Amoeba coli* and he was able by rectal injections of the faeces containing this organism to produce dysentery and ulceration of the lower portion of the large intestine of a dog.

Nevertheless in spite of these and other investigations the general recognition of a specific infection of the intestine due to amoebae came only in comparatively recent times.

Lewis in 1870 and Cunningham in 1871 while studying cholera in India found amoebae in the dejecta of about 20 per cent of the cholera patients examined and even in the stools of healthy individuals. They hence inclined to the belief that these organisms bore no causal relation to intestinal disease. Indeed these investigations and those of Grassi (1879-88) Casagrandi and Barbagallo (1897) and others threw a grave

States Public Health Service show that it embraced in the neighborhood of 1400 reported cases more than 350 in Chicago and 52 deaths. However two thirds of the cases became apparent in cities other than Chicago in fact 206 other localities. Nevertheless the origin of the large majority was traced to the water supply of two hotels in that city as the probable source of infection.

In New York City a search was made by Olesen and Rosenbluth for cases of amoebic dysentery following this outbreak and from November 1 1933 to September 30 1934 11 months 121 cases were reported. However nearly 40 per cent of these cases of amoebic dysentery could not be credited to infection acquired in Chicago. Some of these apparently originated in New York City others elsewhere in the United States than Chicago and New York City and the remainder in foreign countries.

In connection with the fire that occurred in Chicago in 1934 in the Union Stock Yards some 60 cases more of amoebic infection in firemen who drank water polluted by human excreta were discovered by Hardy and Spector.

Carriers of *Endamoeba histolytica* have been widely distributed and relatively numerous in the United States. According to Craig (1937) of 57 561 persons examined in 26 different surveys the average number found to be positive for this parasite was 0.2 per cent. Craig believes that approximately 5 to 10 per cent of the people of the United States are infected with *Endamoeba histolytica* although in some localities the incidence is less than 2 per cent.

## ETIOLOGY AND EPIDEMIOLOGY

The systematic position among the protozoa of the amoebae infecting man is shown in the table (p. 12). They are classified in the Sarcodina in the sub class Rhizopoda.

**Morphology**—The amoebae are unicellular parasites which differ considerably in appearance in the vegetative or trophozoite stage and in the cystic stage. In the former the living motile amoebae possess an endosarc and ectosarc which can usually readily be distinguished when the organism is in motion. The parasite moves by means of pseudopod-like blunt processes consisting of the ectosarc are first protruded and into these protrusions the protoplasm of the endosarc appears to flow.

A great many species of amoebae are found living within the bodies of animals of all kinds chiefly within the digestive tract and frequently give rise to no disturbance. It is therefore essential to have some knowledge of the classification and nature of these different organisms.

Lösch in 1875 gave the name *Amoeba coli* to the species which he found in the human intestine and this organism came to be placed in the genus *Amoeba* (Ehrenberg) 1833. In 1879 Leidy established the genus of *Endamoeba* for the parasitic species found in the common cockroach and named it *Endamoeba blattae*. In 1897 Casagrandi and Bragallo after a study of human intestinal amoebae established the genus of *Entamoeba* for these organisms and named the species they encountered *E. m. ba hominis*. Quincke and Roos Kruse and Pasquale Celli and Fiocca and the writer all recognized from morphological differences from clinical observations and especially from experiments upon animals that more than one species of entozoic amoeba occurred in man and these species were described under a variety of names. Only from a zoological standpoint the evidence of the plurality of species was not entirely complete.

Schaudinn in 1903 supplied zoological descriptions of these two species of amoebae which he described in detail. One of the occurring commonly as a harmless inhabitant of the intestine and not as a parasite he named *Entamoeba coli* and the second species a parasitic one he designated as *Entamoeba histolytica* as the name suggested the ability to dissolve tissues. This second species was regarded as the cause of amoebic dysentery and amoebic liver abscess. In adopting the terminology of *Entamoeba coli*

The disease is also not uncommon in Italy and other parts of southern Europe. In the United States amoebic dysentery is an important disease in the southern states but it is not rare in the northern ones. The practitioner should be on the lookout for cases of the disease in temperate climates generally not only on account of the fact that foreign travel in the tropics has become more common and that carriers of the disease are more frequent but because cases of amoebic dysentery have occurred in the northern United States and in Great Britain, France and other portions of northern Europe in individuals who have not been outside of these localities.

Statistical data regarding the incidence of amoebic infection among different races and in different parts of the world increased materially as a result of the World War. For instance of 31 000 British troops returning to England from the Near East the majority of whom had had dysentery or other intestinal disturbances 9.8 per cent were found infected with *Entamoeba histolytica*. Of nearly 7 000 troops and civilians without any history of bowel trouble examined in the eastern Mediterranean area or invalided from that region 10.5 per cent were found infected. In 5 000 persons with a record of intestinal disorders examined in France and England and constituting mostly troops from the western front 8.9 per cent were found infected while in 3 761 individuals without bowel troubles 5.8 per cent were infected. Kofoid found 10.8 per cent of 1 200 American soldiers returning from France infected with amoebae. This brief summary will give some idea of the spread of sources of amoebic infection due to the war. Of the approximately 50 000 persons examined both in western Europe and the Near East both healthy and dysenteric 13.25 per cent were infected with *Endamoeba histolytica*.

Sapero and Johnson (1939) have made an important study of the men of the U. S. Navy with reference to their infection with amoebiasis. They point out that some 10 000 men are stationed in parts of the world where amoebic dysentery is highly endemic so that the chances of acquiring infection would appear to be considerable. The majority of the men were examined in Panama but as a control naval recruits were examined in Norfolk Va. U. S. A. It was found that recruits from the southern states gave a higher infection rate with *E. histolytica* (14.7) than in the northern states (7.8 per cent).

From these examinations it seemed clear that many of the infections found in men who had served for some time in the Navy had been acquired before the commencement of naval service. For the examination of the men who had been stationed in Panama and repeatedly exposed to infections on shore revealed an incidence of infection of 9.5 per cent which is slightly lower than the 11 per cent found among the recruits. Hence in spite of the exposure in Panama probably no new infections of moment had occurred.

An examination of a submarine group also showed that there had been no spread of infection during the close contact of infected and uninfected individuals which is inevitable in this type of ship.

A different result was obtained with men who had returned from duty in Peking, Shanghai and the Philippine Islands. Here an *E. histolytica* rate of 26.1 per cent was obtained an indication that fresh infections had been acquired in Asia.

The importance of the disease in the northern United States was especially emphasized by the outbreak which occurred in Chicago in 1933 in connection with the Century of Progress Exposition. A committee appointed to study this outbreak and subsequent reports of the United





and *Entamoeba histolytica* for the human entozoa Schaudinn maintained that the description of Lösch was incomplete and that the genus *Entamoeba* established by Cassagrandi and Barbagallo according to the law of priority must be accepted. As the species *Entamoeba hominis* was however probably identical with that of Lösch (*Amoeba coli*) it should be correctly designated as *Entamoeba coli*. He distinguished the 2 species by structural characteristics and showed that with *Entamoeba coli* encystment takes place with the formation of 8 nuclei from which infection occurs. In *Entamoeba histolytica* on the other hand reproduction occurred by binary fission or asexually by peripheral budding in which small aggregations of chromatin reached the periphery of the cytoplasm and enclosed in a resistant capsule broke off from the parent amoeba and constituted the infecting stage. Subsequent investigation threw doubt upon the life cycle of this species as described by Schaudinn and demonstrated his descriptions to be in many respects incorrect.

In 1907 Viereck described a species of amoeba occurring in 2 cases of dysentery in India which he named *Entamoeba tetragena*. This species was shortly afterwards described by Hartmann in 11 cases of dysentery from Africa and was subsequently found to have a wide geographical distribution both in tropical and temperate climates. It undoubtedly appeared to be the usual species encountered in cases of human amoebic dysentery. The sporocysts developed only 4 nuclei instead of 8 as in *Entamoeba coli*.

Subsequent investigations however by Hartmann, Craig, Whitmore, Darling, Wenyon, Walker and others finally led to the abandonment of certain of Schaudinn's erroneous descriptions regarding *Entamoeba histolytica* and to the determination that *Entamoeba tetragena* and *Entamoeba histolytica* are identical. These investigations appeared again to limit the human intestinal amoeba to 2 species *Entamoeba histolytica* commonly with 4 nucleated cysts and *Entamoeba coli* usually with 8 nuclear cysts. However subsequently three other distinct species of amoeba have been found in man. The 5 species may be differentiated by the following characteristics.

### THE HUMAN SPECIES OF AMOEBA

*Entamoeba histolytica* (*Entamoeba histolytica*) as it occurs in the tissues and in the faeces in acute dysentery is usually 20-30  $\mu$  in diameter. The forms however vary in size in different races or strains. Unstained it has a homogenous greyish translucent finely granular endosarc and a clear hyaline highly refractile ectosarc which is best seen in the pseudopods. The single delicate vesicular nucleus is barely distinguishable or usually quite invisible. The organism is actively phagocytic and unlike the saprophytic amoebae frequently contains red cells and occasionally tissue fragments but never bacteria or food particles or (except old or degenerated organisms) vacuoles. In a perfectly fresh preparation on a warm stage it shows active motility moving rapidly across the field usually in a definite direction in a manner which Dobell and O'Connor have compared to that of a slug travelling at express speed. The protoplasm appears to flow across the field without much preliminary extrusion of pseudopods. The other amoebae as a rule are much more sluggish and do not show such directional movement. *E. coli* however may show similar motility. This activity subsides after a few minutes but for some time the organism continues at intervals to throw out abruptly large blunt blade like pseudopodia of clear hyaline ectoplasm. They are very susceptible to chilling or overheating. They quickly round up then and die and can no longer be identified with certainty. The organisms in this free living stage are known as trophozoites or less appropriately as vegetative forms. They are found often in large numbers in the dysenteric stools especially in the blood tinged mucus in scrapings from the base of the ulcers (obtained by proctoscope) in the tissues around the ulcers in the walls of the liver abscesses and (inconstantly) in the pus or discharge from these abscesses. They multiply in the tissues by fission.

## PLATE IV

## Characteristic Forms of the Intestinal Amoebae and of the Three Common Intestinal Flagellates

(From wet fixed faecal smears stained with iron hematoxylin on slide at the U. S. Naval Medical School  $\times 1500$ )

Fig. 1 *Endamoeba histolytica* large race from a case of acute amoebic dysentery. Note the large size of this trophozoite (60  $\times$  12 microns) and the ingested red blood cells some of which have been partially digested.

Figs. 2-6 *E. histolytica* large race from a non-dysenteric case size about 2 microns in diameter. Fig. 2 a trophozoite note the smooth endoplasm without inclusions and the small karyosome in the nucleus. Fig. 3 an immature uninucleate cyst note the large amount of chromatoid matter and the large glycogen vacuole. Fig. 4 an immature binucleate cyst note the fusing of the chromatoid matter into rods. Fig. 5 a mature quadrinucleate cyst with two characteristic rod-shaped chromatoid bodies. The glycogen has been absorbed. Fig. 6 a mature cyst without chromatoid bodies. Note position of nuclei paired and peripheral.

Figs. 7-11 *E. histolytica* small race size about 8 microns. Fig. 7 a trophozoite note close resemblance to the large race trophozoite (Fig. 1). Figs. 8 and 9 are immature and Figs. 10 and 11 mature cysts. Note abundance of chromatoid bodies.

Figs. 12-16 *Endamoeba* sp. Fig. 12 a trophozoite compare with the trophozoites of *E. histolytica* and note vacuolated endoplasm with bacteria and food inclusions. The clumping of nuclear chromatin and the large karyosome. Fig. 13 an immature binucleate cyst compare with the immature cysts of *E. histolytica* (Figs. 3 and 4) and note the diametral position of the nuclei crowded against the cyst wall by the distinctively shaped glycogen mass and the warty granular chromatoid matter. Fig. 14 a mature eight nucleate cyst with typical splint rod chromatoid bodies and nuclei bunched centrally. Fig. 15 another mature cyst showing also the equally characteristic filamentous chromatoid matter. Fig. 16 a mature cyst without chromatoid bodies note again the central location of the nuclei.

Figs. 17-18 *Endolimax nana*. Fig. 17 a trophozoite note the large karyosome and the absence of chromatin granules on the nuclear membrane. The endoplasm is vacuolated as in *E. sp.* Fig. 18 a mature four nucleate cyst nuclei have the same structure as in the trophozoite but the karyosomes are small.

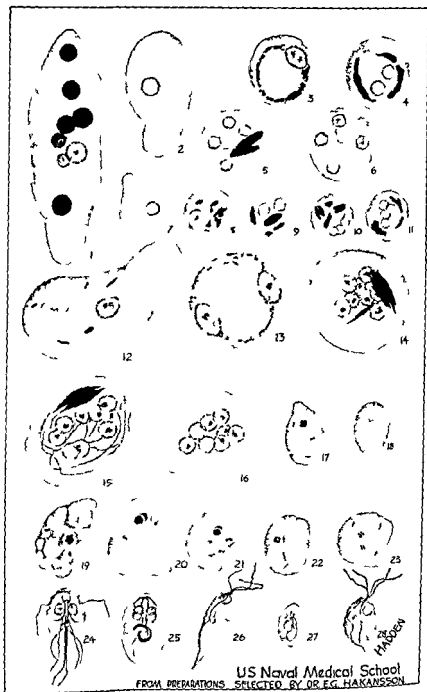
Figs. 19-21 *Iodamoeba butleri*. Fig. 19 a trophozoite note the close resemblance of its nucleus to the nucleus of the *E. nana* trophozoite. Fig. 20 a cyst with distinctive bizarre shape and Fig. 21 a rounded cyst. Both show the characteristic nucleus the glycogen vacuole and groups of granules in the cytoplasm.

Figs. 22-23 *Dientamoeba fragilis* a uni and binucleate trophozoite (no cysts of this species have been found) the nucleus has a distinctive karyosome consisting of granules variously arranged.

Figs. 24-25 *Giardia lamblia*. Fig. 24 a trophozoite (flagellate form) Fig. 25 a cyst. Both have readily recognized morphological features. The trophozoite is rarely seen in the faeces.

Figs. 26-27 *Chlamydomonas*. Fig. 26 a trophozoite (flagellate form) and Fig. 27 a cyst. Note the spiral groove of the nucleus and flagella in the former and the nucleus and variously curved fibrils in the latter.

Fig. 28 *Trichomonas hominis*. A trophozoite no cysts have been found. The wavy line to the left indicates the undulating membrane which is the most characteristic structure of this flagellate.



US Naval Medical School  
FROM PREPARATIONS SELECTED BY DR. E. G. HAKANSSON

## KEY TO GENERA AND SPECIES OF AMOEBÆ (DOBELL AND O'CONNOR)

- ( ) One nucleus present in active amoeba 2  
 (b) Two nuclei present Genus *Dientamoeba* 6  
 (a) Nucleus with small spherical karyosome and peripheral layer of fine chromatin beads Genus *Endamoeba* 3  
 (b) Nucleus with large irregular eccentric karyosome and no peripheral chromatin granules Genus *Endolimax* 4  
 (c) Nucleus with large central spherical karyosome surrounded by a layer of achromatic granules Genus *Ictamoeba* 5  
 3 ( ) Ripe cyst 4 nuclei glycogen diffuse large chromatoids generally present *E. histolytica*  
 (b) Ripe cyst 8 nuclei glycogen in early stages only large chromatoids occasionally present but often absent *E. coli*  
 4 Ripe cyst 4 nuclei glycogen rarely present chromatoids absent *E. nana*  
 5 Ripe cysts 1 nucleus glycogen in dense mass no chromatoids *I. butschlii*  
 6 Nuclei with central granular karyosomes and no peripheral chromatin (Cysts unknown) *D. fragilis*

## COPROZOIC AMOEBÆ

In addition to these a number of other doubtful or rarely encountered species of intestinal amoebæ have been described as well as a number of definite species under other names but which are really identical with the species already referred to. Also a number of free living non parasitic species have been cultivated on artificial media from human faeces which have either been ingested and passed through the digestive tract in this encysted condition or which have been deposited in the stools after they have been passed and have emerged from their cysts in the cultures.

Thus Wells found that in India amoebæ of at least 2 types were commonly present in the air and sometimes found their way into the stools even when the latter had been carefully collected. These amoebæ for the most part are of the *Limax* type and while they have been frequently confused with the human *Endamoeba* they can be distinguished from them at least usually by certain differences in the life cycle such as the type of utrogamy or by the presence of a contractile vacuole. In 1912 Chatton and Lalung Bonnaire created the genus *Vahlkampfia* which includes practically all amoebæ cultivable upon an agar medium and so isolated. Several other species of amoebæ have been reported as pathogenic for man especially by Kofoid and Swezy Faust and Brumpt Brumpt naming his species *Entamoeba dispar*. These are now regarded generally as race of *Endamoeba histolytica*.

Nomenclature—Craig who has carefully studied since 1905 the parasitic amoebæ of man and their classification emphasizes that the generic term of *E. amoeba* originally given by Casagrandi and Barbagallo (1895) to an amoeba found in the intestine of man should be changed to *Endamoeba* established previously by Leidy in 1878 to include the parasitic amoeba of the cockroach *Blattella orientalis*. However some European authorities still retain the name *Entamoeba* and maintain that *Endamoeba blattæ* is not congeneric with *Entamoeba histolytica* and *E. amoeba coli*. However as this has not been demonstrated beyond question and as the International Commission on Zoological Nomenclature has ruled that the name *Endamoeba* is the proper generic one Craig believes that it should be used in preference to *E. amoeba*. This explanation of nomenclature seems necessary in account of the confusion in the spelling of the generic name in different textbooks and articles on the subject of amoebiasis.

Cultivation—The free living amoebæ were grown in earlier years upon a great variety of media in association with bacteria. The most successfully employed were those which did not offer conditions so favorable for the growth of bacteria that they would entirely overgrow the protozoa. Musgrave and Clegg employed for this

*Endolimax nana* (Wenyon and O Connor 1917) is the commonest of these amoebae being reported by Kofoid in 28 per cent and by Dobell in 33 per cent of soldiers examined by them. Faust (1936) in New Orleans found 19.3 per cent of the white race infected, and Meleney in Tennessee 11.9 per cent of the population infected. It is small usually (6 to 12 $\mu$ ) and contains a single small (2 $\mu$ ) nucleus in which the chromatin is largely clustered in a single coarse irregularly shaped karyosome the *lmax* type of nucleus. In fresh preparations it is sluggishly amoeboid and may contain food vacuoles. The cysts are the same in size and contain 4 minute nuclei but no chromatoid bodies or glycogen masses. They resemble the small strains of *E. histolytica* cysts except in the character of the nuclei.

*Iodamoeba bütschlii* (Provazek 1912) (*I. wilhamsi*) measures 8-20 $\mu$  resembling small forms of *E. coli* in its sluggish motility and granular cytoplasm which contains food particles. The single small nucleus has the chromatin largely concentrated in a central karyosome. In films stained with iron haematoxylin they resemble *E. nana*. The trophozoites are much rarer than the cysts. The cysts iodine cysts 8-12 $\mu$  in diameter are oval or irregular and contain a single small nucleus a large compact mass of glycogen but no chromatoid bodies. Although it is believed to be harmless and not to invade the tissues it is highly susceptible to emetine treatment unlike *E. coli* and *E. nana*. Although its distribution is extensive it is relatively rare in many localities. Manson Bahr states that it is found in about 5 per cent of faeces most commonly in those who have been in the tropics and not infrequently in association with *E. histolytica*. Hegner and Taliaferro however report an incidence of 10 per cent to 15 per cent. In the different surveys made in the southern United States it has been found in from 0.25 to 5 per cent of those examined. However Kofoid and James found 21 per cent infected of 367 individuals examined in Colombia South America. It occurs in monkeys and is common in hogs.

*Dientamoeba fragilis* (Jepps and Dobell 1918) is a relatively small amoeba 4-12 $\mu$  in diameter. Often it does not progress and its motility consists of the extrusion and withdrawal of clear fingers of ectoplasm. There are two nuclei present in most instances but as many as 40 per cent have been observed with but one nucleus. The arrangement of the nuclear chromatin may be characteristic and diagnostic. The chromatin is often arranged in a ring of granules 4-5 or 6 in number about midway between the central point of the nucleus and the extremely fine nuclear membrane. Occasionally a very fine dot can be made out in the center of the nucleus which may be a karyosome. When stained with iron haematoxylin if the process of differentiation has not been carried sufficiently far the nucleus may appear to have a large solid karyosome. These amoebae are often difficult to fix and stain properly. Boun's fixative has been recommended as giving better results than Schaudinn's fixative. Cysts are not definitely known. Although this amoeba is wide spread it has been considered rare. However Wenrich et al (1935) reported finding the parasite in 4.3 per cent of 1060 cases University of Pennsylvania students. Sapero and Johnson in the examination of 129 naval recruits in the southern states for intestinal parasites found 63.6 per cent infected with species of amoebae of which 17.1 per cent were infected with *D. fragilis*. This reveals the prevalence of this organism in some localities. Cases have been observed by Gupta in Calcutta (1936) and Hakanson (1936) in Panama. As the organism often disintegrates rather rapidly in the faeces and is relatively difficult to stain it is doubtless often overlooked. It has been regarded as non pathogenic but a few cases have been observed in which it appeared to be the cause of a dysentery and further study of its pathogenicity is needed. Wenrich (1936) has cited several cases with serious gastro intestinal disturbances. Hakanson's case was that of a physician who suffered from acute colitic irritation and diarrhoea of 14 days duration. The symptoms became severe on 3 occasions. He regarded the parasite as perhaps responsible for the symptoms.

*Endamoeba gingivalis* is a (probably) harmless saprophyte living in the mouth especially in pyorrhoeal pockets. It is a sluggishly motile organism 10 to 25 $\mu$  in diameter with sharply differentiated ectoplasm and endoplasm which contains many inclusions (food particles etc). The nucleus is small (2.5-3 $\mu$ ) vesicular with a distinct nuclear membrane and a deeply staining karyosome. Cysts are probably not formed.

containing cysts or dysenteric stool) is rubbed up in the overlying solution and incubated 24 to 48 hours. The sediment is examined microscopically for amoebae. Growth is influenced by the contaminating bacteria, some favoring others tending to inhibit it. By subculturing at 2 to 4 day intervals strains have been maintained for long periods and retain their infectivity for kittens and monkeys. Under favorable conditions encystment (and excystation) occurs.

Snyder and Meleney (1941) have obtained excystation of bacteria free cysts of *E. histolytica* in a medium containing only inorganic salts (modified Locke's solution) but continued cultivation without bacteria was not obtained.

**Pathogenecity of Intestinal Amoebae.**—The presence of amoebae in the dejecta of healthy individual has long been recognized and has previously misled many authors to believe that all amoebae are without etiological or pathological significance. Schüßberg in 1893 found amoebae in the stools of 10 out of 20 healthy persons to whom a dose of Carlsbad salts had been given. Friese and Pasquale when perfectly healthy observed amoebae in the human faeces and in those of 38 persons either healthy or suffering with diseases other than dysentery. Upon some of the who succumbed fresh autopsies were performed and the intestines carefully examined and found to be normal. In 1899 Musgrave and the writer found that 4 per cent of the patients examined in the Philippines who had no dysentery or history of the disease harbored amoebae. In Manila as high as 70 per cent of the healthy American soldiers were found by Ashburn and Craig to harbor *Endamoeba coli*. From its frequent occurrence in healthy individuals for very long periods of time without symptoms of diarrhoea and dysentery and from the negative results obtained from it in animal experiments *Endamoeba coli* has generally come to be regarded as a harmless commensal of man. Later the tendency became prevalent to classify all amoebae found in the stools and intestines of apparently healthy individuals as *Amoeba coli*. This led in turn to considerable confusion until it was recognized that there are periods in the course of amoebic dysentery and particularly in the earlier stages in which symptoms are entirely lacking. Hence when *Endamoeba histolytica* is found in the stools of healthy individual we cannot say that they are not suffering with an early or latent form of the disease or that the malady does not exist in the incubation period unless we follow them over long periods of time in whom no disease develops. In other areas of infection with this amoeba the infection may be of so mild a character as to produce practically no symptoms unless the resistance of the patient is lowered by other intercurrent disease. It is not necessary to suppose in these cases that lesions of the intestine are always present although in some lesions may exist at which we cannot always be aware of during life.

In this connection it is of interest to note that Hegner (1934) has shown that in the study of Philippine monkeys who were carriers of *E. histolytica* and which were subsequently killed no lesions whatever were found in the intestine either macroscopically or microscopically. A somewhat similar observation was also made by Dobell and others.

Johnson (1941) who studied 21 monkeys (7 *Ateles* and 4 *Macaca*) naturally infected with *Endamoeba histolytica* found the large gut in 6 negative for lesions of the intestine by macroscopic examination but in the microscopical examination of the tissues 7 of the 10 showed superficial lesions. These lesions were so small that in most instances they were only discovered when the oil immersion lens was used in examining the stained sections. It is unreasonable to suppose that the same condition may not sometimes occur in human beings and that if autopsies were performed on many human beings who had harbored cysts of amoebae during life there might be a high percentage with no intestinal lesions.

Since the above was written Faus (1941) has reported autopsies on 203 cases in which sudden accidental death occurred and in which the patients had not been observed during life. Only cases were selected in which the autopsy was performed within 4 hours after death. Evidence of infection with *E. histolytica* was discovered in 13 or

purpose agar agar 20 grams sodium chlorid and extract of beef each 0.3 to 0.5 gram prepared as ordinary bacterial agar and with a final reaction of 1 per cent alkali to phenolphthalein Schuckman (1920) has cultivated free living amoebae on an agar media (20 parts of agar 100 parts bouillon and 900 parts distilled water) the amoebae being nourished by bacteria killed by chloroform vapor However it has not been possible to cultivate species of *Endamoeba* upon such media The cultivation of *E. histolytica* upon artificial media was probably first accomplished by Culter (1918) In that year he reported the successful cultivation of *Endamoeba histolytica* on an egg medium to which was added a few drops of blood

However Dobell was not able to confirm cultivation upon this medium In 1924 25 Boeck and Drbohlav demonstrated for the first time in a convincing way the successful cultivation of this organism They employed a Locke-egg medium prepared as follows

Four eggs are washed brushed with alcohol and broken into a sterile flask containing glass beads Fifty cc of Locke's physiological solution are added and the mixture broken up by shaking Test tubes are then filled with a sufficient quantity to produce slants from about 1 to 1½ inches upon coagulation by heat These tubes are now slanted in an inspissator and heated (70 C) until the egg mixture has solidified They are then transferred to the autoclave and sterilized for 20 minutes at 15 pounds pressure

The tubes are now covered to a depth of 1 cm above the egg slant with a mixture composed of 8 parts of sterile Locke's solution and one part of sterile inactivated human blood serum They are then incubated to determine sterility

#### LOCKE'S SOLUTION

Distilled water	1000.00 cc
NaCl	9.0 Gm
CaCl <sub>2</sub>	0.2 Gm
KCl	0.4 Gm
NaHCO <sub>3</sub>	0.2 Gm
Glucose	2.5 Gm

(2) *Locke egg albumin or L E A medium* which is prepared by covering the egg slants with Locke's solution containing 1 per cent of crystallized egg albumin It has the advantage over the L E S medium of being more readily prepared since the albumin is usually more available than human serum

The best growth of amoebae occurs between pH 7.2 and 7.8 which is usually the pH of the L E S and L E A media Adjustment however may be required

With cultures of *E. histolytica*, on their medium Boeck and Drbohlav successfully inoculated cats and produced dysenteric lesions in the intestine Cleveland and Collier found that they were not able to obtain a really satisfactory cultivation of *E. histolytica* until they employed slants of liver infusion agar

**Cleveland and Collier's Medium (1930)** — (1) Liver infusion agar (Difco dehydrated) 30 Gm (2) Disodium phosphate 2 Gm (3) Distilled water 1000 cc Autoclave and slant Cover the slants with a 1 in 6 dilution of sterile fresh horse serum in physiological salt solution and add a 5 mm loop of sterile rice flour or powdered unpolished rice In making subcultures remove 2 or 3 drops of the rice flour debris from the bottom with a sterile wide mouth glass pipette These authors reported obtaining *E. histolytica* in nearly every case despite bacterial contamination

Gladys Craig (1939) has found that the addition to Cleveland's medium of Difco tryptone and Difco yeast extract accelerate the growth of amoebae

At the National Institute of Health favorable results have been obtained with Boeck's egg slant overlaid with Ringer's or Locke's solution to which is added 250 mg of dehydrated Löffler's blood serum per liter A loop full of faeces (either solid faeces

these lesions. However he has found no evidence that the amoebae produce a special toxin or toxins that cause the symptoms.

Brumpt has reported that inoculation of a cat with the cysts of *Endamoeba coli* and *E. dispar* in association from a human case resulted in the formation of small intestinal ulcerations containing the amoebae. These findings suggested that *E. coli* under certain conditions might become pathogenic for man since in his hands *E. dispar* does not give rise to intestinal ulcerations in cats. However others have thought that *E. dispar* is a strain of *E. histolytica*.

We know little yet as to whether the pathogenesis of some species of amoebae under certain conditions may be increased by long periods of existence in the human intestine.

Whether or not *E. histolytica* will produce intestinal lesions in an individual is probably dependent to some extent upon the environment which the protozoa find in the intestine. The hydrogen ion concentration of the intestinal contents and bacterial flora, the previous existence of intestinal lesions, the resistance of the host and possibly the presence of specific or non-specific substances in the blood are all factors which may influence at different times the pathogenic action of the amoebae.

Vogel found in 49 cases of infection with *E. histolytica* (dysentery) that a haemolytic Gram positive diplococcus differing immunologically from haemolytic streptococcus was present in 44. Intestinal lesions were produced in animals inoculated with cultures of this organism. He believes that this diplococcus is a contributory factor in the pathology of amoebiasis.

Also the presence of certain other bacteria may influence the pathogenicity. Nauss and Sabinger (1935) found that in kittens a strain of *E. histolytica* failed to infect them unless a haemolytic culture of *Bacillus coli* was also injected with it. Unquestionably the chemical environment in the intestine exerts an effect upon the life activity, pathogenicity and encystment of *E. histolytica*. Chang (1935) in the hydrogen ion concentration resulting in increased alkalinity cause *Endamoeba* to encyst (Knowles, Napier and Das Gupta 1935). They found that in dysenteric stools with trophozoites that the pH was in the neighborhood of 6.2 and in stools in which the amoeba had encysted the pH was between 7.5 to 8.18.

Gladys Craig (1936) has found that the growth of *Endamoeba* is heavier in a buffered medium than in an unbuffered one and that encystment occurred with a pH of 7.2 to 8.3 corresponding rather closely to the observations of Knowles and his associates.

Faust and Kagy (1934) have found that in experimental amoebiasis of the dog feeding raw liver to dogs was beneficial in arresting the amoebic process as in certain cases this produced complete eradication of the pathogenic organism as the amoeba became encysted. In 1935 they found that intramuscular injections of the liver extract did not eradicate the amoebae.

Meleney and Frye (1933-39) believe from experiments on animals the power of amoebae to invade the tissues may depend upon the pathogenic index or virulence of the strain.

Cleveland and Sanders (1930) and Cleveland and Collier (1930) think that the bacterial flora has a great deal to do with the growth and encystment of amoebae and the pathogenic effect. They have studied the virulence of several strains of *E. histolytica* for the liver of cats and have found that when the amoebae are grown in culture and a uniform inoculum is made in the liver some strains produce a much higher percentage of liver abscesses than others suggesting a variation in virulence. But when cultivated for a year or more the amoebae appeared to lose their pathogenicity for the liver and intestine of the cat. However they demonstrated that it was the bacteria growing with the amoebae that had lost virulence for when the virulence of the bacteria was regained by liver passages the amoebae that had apparently lost their pathogenicity were again capable of producing a high percentage of abscesses when



6.44 per cent and amoebic lesions were demonstrated in 5 of the 13. In these 5 and possibly in 2 more there was concrete evidence of tissue invasion by the amoebae. The lesions were superficial and confined exclusively to the mucosa.

Some observers have believed that whenever *E. histolytica* is present in man it gives rise to more or less severe intestinal lesions. Dobell among others suggested this but there is not always proof that such is the case. Others think that many persons who only pass cysts of *Endamoeba histolytica* may have no symptoms of disease and no intestinal lesions. Hakansson points out that the cysts in the intestine which survive develop into trophozoites. These subsequently divide and so bring about an increase in the amoebae which may remain as trophozoites or become encysted. He thinks the so called cyst carrier will pass trophozoites from time to time when the stools become more liquid. A few formerly inclined to the belief that all amoebae found in the intestine are or may become pathogenic and they maintained that in every case where human infection was found even though there be no symptoms treatment should be insisted upon until the amoebae disappear.

Rothman and Epstein (1941) have again advanced these views and believe that all the amoebae that occur in the intestine may be pathogenic but they have not demonstrated that fact and their views have not been concurred in by others.

**Carriers**—The recognition of the carrier condition in connection with *Endamoeba histolytica* formerly observed in various other infectious diseases has also assisted in clearing up many difficulties in understanding the nature of this parasite. These amoebic carriers may be divided into contact carriers and convalescent carriers the former having never suffered from amoebic dysentery the latter having had an attack of amoebic dysentery and having recovered from a clinical standpoint the infection with amoebae persisting.

The contact carrier therefore may represent a healthy individual whose infection does him no appreciable harm while the convalescent carrier constitutes an individual who has shown himself susceptible to the action of the parasite and who continues to pass cysts of this parasite in his stools.

The percentage of persons who acquire infection with *Endamoeba histolytica* and subsequently become healthy carriers cannot definitely be stated. The figures of Walker and Sellards show that of 18 men experimentally infected with *Endamoeba histolytica* only 4 (22.2 per cent) developed symptoms of amoebic dysentery. The rest 14 or 77.8 per cent became contact carriers. Some of them were under observation for over 2 years and never showed any signs of dysentery or other amoebic disorders. Wenyon and O'Connor found 106 carriers among 1,979 healthy men examined in Egypt of these 106 infected individuals only 16 gave any history of dysentery and they believed the latter figure too high for the diagnosis of amoebic dysentery for in no case were they certain of the type of the disease from which the individual suffered. Dobell and Meleney (1935) believe that no more than 10 per cent of persons who become infected with *Endamoeba histolytica* suffer to any appreciable extent from their infections.

In regard to the incidence of actual amoebic dysentery in the U.S. Navy (1939) with 15,000 carriers of *E. histolytica* there were only 4.6 cases for every thousand carriers during the course of a year and Saperio points out that it is yet to be shown what are the determining factors necessary for the development of actual amoebic dysentery in apparently healthy carriers.

However Craig believes that at least 50 per cent of carriers of *Endamoeba histolytica* suffer from symptoms due to the presence of the parasite. The most common symptom noted is constipation with occasional attacks of diarrhoea usually mild but sometimes accompanied by pain. Another common symptom is loss of appetite sometimes amounting to actual distaste for food this is accompanied by loss in weight. Other symptoms noted are tenderness on deep pressure in the right iliac region and over the descending colon dull pain in the lower abdomen flatulence and abdominal distention sometimes tenderness over the liver neuralgic pains in the lower abdomen. A mild degree of anaemia is usually present evidenced by a slight pallor. Many carriers also present symptoms referable to the nervous system of the neurasthenic type. The symptoms are due the author believes to definite lesions of the intestine produced by the amoebae and to the absorption of bacteria and toxins from the intestine through

lating the material directly into the caecum they were able to infect every one of the kittens and were also able by this method to propagate a strain of amoeba through a series of animals for several months. In their hands the intracaecal inoculations yielded positive results in the diagnosis of human amoebiasis when the clinical manifestations were obscure and the amoebae in the discharges so few and atypical as to make such an examination unsatisfactory. Amoebic abscess of the liver has resulted in both cats and dogs as a sequel to experimental intestinal infection in a number of instances. Natural amoebic intestinal infection and spontaneous liver abscess has also occurred in monkeys.



FIG. 17.—Photograph of middle and upper portion of the large intestine of a cat. In the vicinity of A may be seen a group of well marked ulcers. A divided with a lymphatic gland appearing on the left of the photograph.

Many other investigators have since inoculated animals with amoebae successfully. Swartzwelder (1939) has infected dogs by feeding orally not only cysts but trophozoites. Excystation occurs in the small intestine but not in the large.

Another method of infection consists of feeding encysted forms of amoebae. Casagrandi and Barbagallo, Calandruccio and Schaudinn fed themselves encysted amoebae apparently of the *Entamoeba coli* type, produced infection and re-obtained the amoebae from their stools though they had no symptoms of disease following the infection. They also infected cats by feeding encysted cultures of this parasite and obtained no symptoms of disease. Both Schaudinn and Quincke and Roos fed cysts of *Entamoeba histolytica* to cats and obtained ulcerations of the large intestine in which numerous amoebae were found.

Walker and Sellards have performed the most important experiments in man in connection with infection by feeding amoebae. They first fed cultures of amoebae cultivated from water or other non-parasitic sources as well as from dysenteric stools to ten men without producing dysentery in a single instance or finding such amoebae in the stools upon microscopical examination. Of 20 such experiments performed on the 10 men however in 13 they recovered amoebae in cultures from the faeces from the first to the sixth day but never afterwards. They concluded that amoebae which can be cultivated are non-pathogenic. Twenty cases were fed with material containing *Entamoeba coli*. There was a uniform failure to recover organisms culturally from the stools and in no instance was dysentery produced though 17 became parasitized as a

inoculated with these bacteria. The experimental work of Deschiens (1938) also suggests that the flora associated with the amoebae are important in determining pathogenicity.

Nauss and Rapport (1940) have performed extensive experiments on cats to determine the influence of certain accessory factors in the initial penetration of the colonic mucosa by *E. histolytica*. They believe that they have demonstrated that irritation of the intestine produced by feeding croton oil and bacteria derived from one of their amoebic stool cultures showed definitely the importance of accessory aids to penetration or positive invasiveness by *E. histolytica* of the colonic mucosa of cats as well as kittens. Deschiens and DeCourt (1938) have also employed croton oil as a toxic traumatizing agent and find that it assists in the penetration of the amoebae into the mucosa.

Westphall (1937) infected himself by swallowing a capsule containing washed cysts from a carrier with *E. histolytica*. Two days later cysts and vegetative forms were seen in his stools in small numbers which later became abundant. After remaining symptomless for 8 months he and a colleague drank 50 cc. each of a thick suspension of an amoebic dysentery stool which had been supposedly free of amoebae as shown by control tests. Both Westphall and his colleague serving as a control for the bacteria developed a mild dysenteric colitis lasting for a few days; the bacteriological examinations showing non-pathogenic bacteria only. However, after 28 days Westphall developed a typical amoebic dysentery while his colleague suffered no more after the first few days of inconvenience.

He concluded therefore that the early bacterial infection lowered the resistance of the intestinal mucosa which rendered it possible for *E. histolytica* to invade the intestine with frank symptoms of amoebic dysentery. Meleney (1935) points out that individuals seem to differ greatly in the susceptibility of the intestines to the invasion of amoebae and not more than 10 per cent of infected persons and in some regions even less than that develop active amoebic dysentery.

Horster from experience in North Africa concludes that *E. histolytica* is in the first place a harmless inhabitant of the intestine and that actually amoebic dysentery only follows when the wall of the intestine has been injured so that infection of the intestinal wall by the amoeba can take place. The commonest cause of such injury is bacillary dysentery.

Spector (1936) found in patients during the Chicago epidemic 2 races of *E. histolytica* in the stools, a large and a small one. The large race given per rectum resulted in typical severe amoebic infection in kittens while similar inoculations of the small race failed to produce results. She thought that in human beings the small race produced milder symptoms than the large race. Frye and Meleney (1938) also believe that a small race of *E. histolytica* exists which possesses low power of invasion of the tissues of man and experimental kittens.

Further knowledge of the means of distinguishing the pathogenic and non-pathogenic amoebae is desirable.

## INOCULATION EXPERIMENTS IN ANIMALS AND MAN

Several methods of infection with amoebae have been employed. Positive results have been obtained in cats and dogs by the direct injection into the rectum of faeces containing the amoebae. While not all of the inoculated animals become infected and single experiments and those in which unsuitable material is employed often fail, nevertheless there is no doubt that amoebic lesions may be produced in the large intestine in cats by this method.

The writer (1900) obtained dysentery and perfectly typical amoebic ulcerations in the large intestine of cats by the injection into the rectum of portions of the contents of a liver abscess which contained living amoebae but was otherwise sterile. These lesions were perfectly typical of those seen in man. Many of the ulcerations showed a distinct undermining of the mucosa and round cell infiltration of the submucosa with numerous amoebae at the base of the ulcers; the experiments were practically conclusive of the pathogenesis of the species of amoeba employed (Fig. 122). Sellards and Baetjer showed that inoculation of kittens by rectum or by feeding dysenteric stools rich in amoebae has resulted in infection in about 50 per cent of the experiments. By inocu-

all cysts immediately in a strength of 1:20 in one minute in a strength of 1:30 one half in a strength of 1:100 and not at all in a dilution of 1:2000. Cresol can obviously be employed safely then for the disinfection of dysenteric stools or the hands of those who have to deal with patients. Acid sodium sulphate tablets and chlorinated lime tablets used in the strength for the purification of water failed to kill the cysts.

The resistance of the cysts to chlorine is a most important question in view of the common use of chlorine in water purification and its efficiency in destroying bacteria in drinking water supplies. Not only Wenyon and O'Connor but Mills, Bartlett and Kessel and Yorke and Adams have all found that drinking water cannot be freed of the cysts by chlorine in reasonably dilute amounts. Certain experiments of Garcia have suggested that the cysts were not killed until the concentration of chlorine reached 3.5 parts per million of chlorine which strength obviously renders water unfit for drinking purposes. Only Stone (1937) has reported that cysts of *Endamoeba histolytica* and cultures may be destroyed by small amounts of chlorine in water and in his experiments the cysts were no more resistant than *Bacillus coli communis* in the cultures.

Possibly the killing of the bacteria by chlorine in these experiments influenced the destruction of the parasites and their development from the cysts. Usually amoebae will not excyst in a culture medium in the absence of living bacteria. Craig (1940) points out that the cysts are very resistant to chlorine and it has been demonstrated that it requires approximately a hundred times as much chlorine when mixed in water to kill them as is used in water sterilization. Chloramine has been found less efficient than chlorine and neither of the  $\epsilon$  agents can be employed to render water safe that is contaminated with the cysts of this parasite. Chang and Far (1941) have carried out careful experiments which show that fairly heavily contaminated water can be disinfected successfully with the practicable and lower range of superchlorination provided the contact period can be extended to 30 minutes or longer.

The results of the destruction of *Endamoeba histolytica* in cultures by emetine are somewhat contradictory. Early experiments by Vedder with ipecac and later by Wherry, Boman and the writer with emetine indicate the toxic effect of this drug in concentrated solutions on cultures of free living amoebae and frequently of the cysts. However Dale and Dobell found emetine not particularly toxic when applied directly to *Endamoeba histolytica*. Yorke and Adams (1927) found in their experiments that the cysts of *E. histolytica* were very resistant both to emetine and yatrien. However Dobell and Laidlaw (1927) found emetine and encephalin were specific poisons for this amoeba. In cultures a strength of emetine of 1 in 5 million was lethal. Encephaline on the other hand was less effective. They found emetine 10 times as poisonous as stovarsol, 50 times as poisonous as quinone. Dale and Dobell however suggested that the specific action of emetine in amoebic dysentery is especially because of its primary action on the host and not directly on the parasite. More recently St John has determined that emetine kills the amoebae from 10 to 50 times more readily when the medium kept alkaline than when it is acid. In a dilution of 1 in a million it killed the amoebae regularly in 2 or 3 days in alkaline medium while in the case of 2 or 3 trials death occurred in 3 or 4 days with a dilution of 1 in 1,000,000. Santel found that emetine in dilutions of 1:50 to 1:1000 killed cultures of *E. histolytica* after 24 hours while solutions of yatrien 1:200 to 1:400 and of stovarsol 1:50 were necessary to destroy the amoebae in this period of time.

The cysts will certainly not withstand the drying of a tropical sun so that it is improbable that wind in blowing about dust can play an important part in the spread of amoebic infection.

#### EPIDEMIOLOGY

**Mode of Infection and Spread**—The transmission of *E. histolytica* to man may occur (1) by contamination of water or food with faecal material containing cysts (2) by droppings of flies and cockroaches (3) by the use of human excrement in the fertilization of vegetable gardens and (4) the spread of infection by lower animals as monkeys and rats. The handling of food by infected individuals is also believed by some to

result of a single feeding in from 1 to 11 days the endameba being found in the stools and persisting there for extended periods. They concluded that *Endamoeba coli* is an obligate parasite, non pathogenic and cannot be cultured.

In another series of experiments 20 feedings were carried out with *Endamoeba histolytica*, the faecal material being mixed with powdered starch or magnesium oxide and given in gelatin capsules. Seventeen men became parasitized after the first feeding, 1 required 3 feedings and 2 who did not become parasitized at the first feeding were held as controls. The average time for parasitization was 9 days. Only 4 of the 18 parasitized men developed dysentery which came on 20, 57, 87 and 95 days respectively after the ingestion of the infecting material. The 4 cases of experimental dysentery resulted from the feeding of material from normal stools of carriers.

**Resistance of Amoebae to Physical Conditions and Chemical Substances**—The behavior of amoebae towards physical conditions and chemical substances is of importance in connection with the prevention and treatment of the disease. The vegetative forms of amoebae usually undergo disintegration in a short time after the stool is passed. In faeces kept at laboratory temperatures 16 to 20 C the cysts also die fairly rapidly in 3 or 4 days and are all dead within 10 days. The cysts also do not appear to withstand drying for any length of time for they stain with dilute eosin at once after drying and when cysts stain in this manner they are probably dead. On the other hand the cysts may survive for as long as a month in water which has been mixed with faeces particularly if the dilution of faeces by the water is sufficient to prevent intensive bacterial growth. The amoebae are usually destroyed by a temperature of 60 C maintained for one hour even when encystment has occurred. In cultures York and Adams found the cysts survived at a temperature of 45 C for 30 minutes but are killed within 5 minutes at 50 C.

The trophozoites usually lose their motility in the stools and quickly die but when encystment has occurred freezing at least of the free living species for as long a period as a month may not destroy them. In cultures cooling immature cysts to 0 to 5 C for 48 hours tends to destroy them or to interfere with their capacity for development though Swartzwelder found that cysts refrigerated for 43 days at about 5 C excysted when fed to a dog.

Considerable work has been done upon the resistance of the cysts of *Endamoeba histolytica* to various chemicals but much of it has been worthless owing to the crude technique employed. For determining the life of the cysts 2 tests have especially been employed first the eosin staining test second the culture viability test. The latter seem to be the most reliable. In testing the effect of bichloride of mercury Kuenen and Swellengrebel using the eosin viability test found that a solution of 1:1000 killed all the cysts and after an exposure of 4 hours. However Yorke and Adams using a culture viability test found that a 1:2500 solution killed the cysts in 30 minutes. In employing formalin Kuenen and Swellengrebel and Boeck using the eosin viability test found no dead cysts were observed after 10 minutes exposure to a 10 per cent solution of formalin and Boeck even found cysts of this amoeba still viable after a 5 day exposure to a 5 per cent solution of formalin. On the other hand Yorke and Adams using the culture viability test found that a 0.5 per cent concentration of formalin killed the cysts after an exposure of 30 minutes. Permanganate of potassium has not been found to be of special value in destroying the cysts except in very concentrated solutions. The action of creosol is much more favorable. Kuenen and Swellengrebel found that by using the eosin test a 1:250 solution killed most of the cysts in from 5 to 10 minutes while Wenyon and O'Connor using the same test found that cresol killed

no treatment at all. If the cysts from such cases find their way into the water supply or into moist food without having been dried, they are likely to give rise to outbreaks of amoebic dysentery.

The transmission of *Endamoeba histolytica* by water is common where there is no properly controlled and filtered water supply, and also where the inhabitants depend upon wells, springs, sluggish streams, and storage tanks for water. Epidemics of amoebic dysentery are usually caused by a polluted water supply. Such outbreaks were not uncommon among the troops in our military operations in the Philippine Islands in earlier years, and were described by Craig and by the writer as early as 1899. Classical examples of epidemics due to an infected water supply in the United States have been the one in connection with the Century of Progress Exposition in Chicago in 1933, in connection with which there occurred some 1409 cases of amoebic dysentery or amoebic colitis, reported by McCoy and Hardy and others in 1936. The epidemic which occurred in Chicago concerned especially the employees and guests of 2 large hotels, among which some 800 cases developed. The infection was traced to an error in plumbing. A direct communication had been inserted between a sewer pipe and an intake pipe supplying drinking water, through which it was possible for a reverse flow from the sewer pipe to take place. Cysts were found in water drawn from the pipe in the vicinity of this communication. Another water-borne epidemic occurred at the Union Stockyards in Chicago in 1934, among firemen who drank water infected with human excreta, from which some 100 cases of amoebic infection occurred. It is probable that water-borne infection is much more common in the United States than was formerly supposed, and we must not presume that other outbreaks will not occur from time to time.

Flies may also give rise to local epidemics of amoebic dysentery. Such an epidemic has been reported by Craig at El Paso, Texas, in which 118 cases of the disease occurred among the troops camped in that city. Flies also are probably of importance in the spread of the disease to individuals. Cysts of *E. histolytica* have been found in large numbers in the faeces of insects (*Musca domestica*, *Fannia canicularis*, *Lucilia*, *Calliphora*). House flies readily take up free and encysted forms in human faeces and can pass them from the gut as early as 5 minutes and as late as 20 hours after feeding. A single house fly may take up one milligram of faeces in one half hour. Wenyon and O'Connor found that wild flies captured in Alexandria often deposit in their droppings cysts of protozoa and eggs of worms which they have evidently taken up from human dejecta on which they have fed. The cysts can readily pass unaltered through the intestine of the fly.

Root (1921) also found viable cysts in the droppings of flies as long as 48 hours after they had fed upon contaminated faeces. Frye and Meleney (1932) found cysts of amoebae in the intestine of flies caught in 4 of 12 houses where individuals infected with this parasite resided. Cockroaches may also be a source of infection of food, since Macfie (1922), Tygera (1926), and Meleney and Frye (1936) have found that cysts of amoebae

be an important source of infection. One of the greatest sources of infection is through drinking water contaminated directly or indirectly with faecal material from cases of amoebic dysentery. Food, particularly when uncooked such as lettuce and other salads may also be a source of infection. The use of human excreta in the fertilization of vegetable gardens is an important means of transmission in some countries, particularly in the Far East. The amoebae which are found almost constantly in the water in some tropical countries and which have been easily cultivated on artificial media are usually of the *Limax* type and are non-pathogenic for man. However in amoebic dysentery the free motile trophozoites of *E. histolytica* are often found in the faeces in large numbers where they may survive for brief periods and these may come into contact with water and food. It is generally believed that only the cysts are infective for man, and that if the trophozoites are swallowed in water or food they are destroyed by the gastric secretions. There is no conclusive evidence that man may be naturally infected by trophozoites. However the trophozoites which have been in some experiments fed to kittens and dogs are not all killed and these animals have been successfully infected in this way. A recent example of this was reported by Swartzwelder (1939) who has successfully infected 5 of 13 dogs with *E. histolytica* fed to them in a medium which was cyst free. Also he demonstrated that the trophozoites passed through the stomach in a viable condition and that they could stand fairly high concentrations of hydrochloric acid *in vitro*. As the acute symptoms of the dysentery abate smaller amoebae occur and many of these become encysted in the large intestine in transparent capsules in which condition they are passed from the intestine in the faeces in very large numbers. The cysts of *Endamoeba histolytica* on account of the capsule are relatively hardy structures which though they also cannot withstand drying will nevertheless survive for considerable periods if they remain moist. If they are kept moist and cool they may in fact, survive for several weeks outside of the body in water or in faeces. The spread of amoebic dysentery generally occurs from ingestion of these cysts in water or food. The cysts usually pass through the stomach and upper portion of the small intestine unchanged. However, in the lower portion of the small intestine the cyst wall becomes softened through the intestinal secretion, and excystation occurs. Ogura (1938) in detailed experiments upon white rats has shown that for excystation another factor is essential besides suitable moisture: temperature, pepsin in an acid media, bile and pancreatic extract. He thinks this additional essential factor is the presence of certain bacteria. Upon excystation each cyst gives rise to a four nucleated amoeba. According to Dobell after a complicated series of nuclear divisions eventually there are produced 8 small amoebae known as trophozoites. These grow into adult forms which may invade the tissues of the large intestine and produce amoebic colitis or dysentery. The cysts are often passed in very large numbers by healthy carriers or by patients who have partially or apparently wholly recovered from amoebic dysentery either after incomplete or ineffective treatment or after

recently emphasized by Spector (1934) and Baylis (1936) but as pointed out elsewhere in this article the cysts cannot be destroyed in drinking water by chlorination as the amount of chlorine required to kill them is much higher than could be used in a public drinking water supply

**Meteorological Conditions**—Meteorological conditions have considerable influence upon the disease which is more prevalent in the summer months May to August inclusive. In the United States Simon found the greatest incidence in the Gulf states during April and May. Hinman and Kampmeier (1937) found no seasonal incidence in New Orleans in the study of 400 cases. However more cases were admitted to the hospital in June. Triebel and De (1938) have found in a study of disease among Europeans in Calcutta from 1929-1937 that while the greatest incidence was in July and August owing perhaps to the habits of life of European they found that the incidence did not always follow the periods of highest rainfall. Among the Indian population there was a more definite seasonal correlation with the disease. The seasonal variation is usually more closely associated with variations in humidity and does not correspond so closely with those of temperature. In the tropics the disease often becomes more prevalent during the rainy season (See Fig. 138). During the season when there is much rainfall there are increased chances of water supplies becoming contaminated with cysts from polluted soil being washed into wells and springs. Flies which may serve to transmit infection also are more prevalent following the rains.

**Age Sex Race**—Infection occurs at all periods of life and if opportunity for contracting the disease is taken into account liability to infection is approximately the same at all ages. The enteric symptoms of the disease are common in children under 10 years of age but are much rarer in children under 5 years of age.

In Fitch's series of 119 cases of amoebic dysentery treated at the Johns Hopkins Hospital 34 were under 10 years of age and 65 contracted the disease between the ages of 10 and 30. Of 200 cases investigated by the writer in the Philippines the disease developed in 149 of the patients when they were between 10 and 40 years. In Malaya the average age of half of the patients treated was from 20 to 30 and in the Andaman Islands the preferential period of life is approximately the same. In 15 cases of amoebic dysentery in the southern United States reported by Musser (1927) 35 occurred between the ages of 10 to 40. After 40 years the case incidence everywhere falls rapidly. It should be borne in mind however that it is mostly between the ages of 20 to 40 that men are exposed in the tropics to the risks of infection and that variations in incidence are due particularly to the opportunity of infection rather than to the period of life.

For the same reason the records of sex incidence cannot be regarded as demonstrating a predisposition although almost all observers agree that the disease is much more prevalent in males. In the Philippine Islands of 401 hospital cases of the disease the ratio of males to females was as 4 to 1 and in 200 personal cases of the writer which were analyzed for statistical purposes only 23 were in females. Haris states that it seems to occur in males about 3 times as frequently as in females while in Fitch's series there were 103 males and 21 females. Hinman and Kampmeier (1937) found that in Louisiana infected males predominated over females in the ratio of 4 to 1.

The returns compiled by government hospitals in the eastern tropics by Carnegie Brothers showed that 5 males to 1 female is an average proportion. However Tao (1932)



will survive in the intestine of cockroaches for as long as 48 hours after feeding on infected material

Tijera reported the production of amoebic dysentery in 2 kittens by feeding of droppings of cockroaches containing the cysts

*Infection from lower mammals*—Certain of the lower mammals may also transmit the infection. In the tropics especially infection of local water supplies such as cisterns may occur from the excreta of monkeys and occasional infection of food by the droppings of rats must be considered

Naturally infected *Macacus* monkeys and orangoutangs were observed by the writer in 1899-1900 in the Philippine Islands with amoebic dysentery. Dobell (1931), and Hegner (1932) have also observed naturally infected monkeys

Lynch (1915) reported the presence of *E. histolytica* in rats in the United States and emphasizes the importance of this amoeba in the spread of the disease. Chiang (1925) and Tanabe and Andrews (1934, 1936) and Boe (1939) have also found it possible to infect rats by oral administration of the parasite. Faust (1930) and Anderson (1932) have observed naturally infected dogs in the United States. Pigs in the United States have not been reported infected but Kessel (1928) reported pigs in China infected with *E. histolytica*

There has been some difference of opinion in regard to the importance of the rat in the transmission of the infection to man. Tsuchiya (1939) has carefully studied this question anew. He found that in feeding cysts to rats one of the factors influencing infectivity was the acid concentration of the gastric juice of these animals and he suggests that similar conditions may occur in man and prevent infection if there is a high concentration of the gastric juice. He points out that his experiments show that cross infection between man and rats is apparently possible and that rats may be conceived as a reservoir for *Endamoeba histolytica* though probably rats are not an important factor in the spread of the disease to man

*Food Handlers*—Some writers think that a common method of transmission of amoebic dysentery is by *food handlers* who are carriers of the infection and there has been considerable discussion as to this means of transmission. The experiments of Spector and Buky (1934) suggested that infection was not likely to occur from the hands of individuals

Andrews however demonstrated that the cysts may remain under the finger nails of individuals in a viable condition for periods varying from 5 to 45 minutes. Craig (1937) believed that the disease is very commonly contracted through infection acquired by infected handlers of food. He points out that the incidence of carriers among food handlers in public eating places has been found to be high in numerous localities

Sapero and Johnson (1939) have recently carried on in naval mess units practical experiments and properly controlled among 14 groups of people comprising in all 919 persons who had been served food and drink by carriers of *E. histolytica*. They found there was no evidence to support the belief that infected food handlers were important agents in the transmission of amoebiasis

Cysts of *E. histolytica* are fortunately removed completely from water by coagulation and filtration through rapid sand filter beds as has been

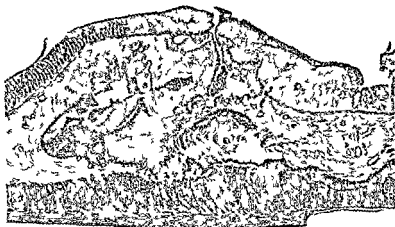


FIG 123—L. to m. body type showing destruction of mu. sa. and und. among the bru. co. (X 10)



FIG 124—Edge of lesion shown in Fig 123. Illustrates reaction in submu. sa. b. w. mu. ova. Note the cytolytic and diagenetic changes in amoeb.

in China in a survey of the rate of infection of 8445 people found infected 670 (or 11.46 per cent) of 5850 males examined and 208 (or 8.02 per cent) of 2695 females examined. In this locality conditions under which women were exposed to infection more closely approximated those under which the men lived.

All races in the tropics who do not take proper precautions are liable to attack. The Malay and black race appear slightly less susceptible than the white race though the predisposition of native races depends considerably upon their habits of life. The better classes of Chinese residents who drink only hot water or tea are more rarely infected. Nevertheless natives who by reason of their mode of life and the condition of their drinking water are very frequently exposed to infection do not suffer so often or usually as severely from the disease as do Americans and Europeans.

### PATHOLOGY

Human infection probably occurs in nature only as a result of the ingestion of cysts.

In the intestine the cysts undergo (metacystic) development each finally giving rise to 4 small trophozoites. These often attach themselves to the epithelium of the large intestine especially in the crypts. They may also penetrate into the tissues of the mucosa and quickly into the submucosa partly by their own active movements and partly by means of a lytic substance which they secrete. Here they often give rise to small areas of gelatinous necrosis (abscesses) which rupture into the lumen of the intestine and produce ulcers. The subsequent course depends upon the balance between the destructive powers of the organism and the reparative powers of the host. In most cases the latter suffice to restrict the lesions to small and even microscopic dimensions and the infection is symptomless. Often however the defensive forces appear inadequate (without treatment) to eliminate the infection entirely, once it is established in the tissues. In other cases the organisms penetrate into the submucosa and extend laterally in this layer, undermining the mucosa and leading to the formation of large ulcers. Secondary bacterial infection from the intestine then occurs. The portions of the bowel wall between the ulcers are commonly not inflamed. The muscular coat is relatively resistant but in the severest cases it may be penetrated as well as the serosa giving rise either to perforation and general peritonitis or to the formation of adhesions to neighboring structures. In severe chronic cases there are extensive adhesions, marked scarring of the intestinal wall in some places with thinning and dilatation, in others with thickening of the wall and narrowing of the lumen and occasionally the formation of tumor-like masses of granulation tissue. A striking feature of all the lesions (intestinal and hepatic) is the absence of leucocytic infiltration unless secondary bacterial infection occurs.

**Morbid Anatomy**—In amoebic dysentery the large intestine is chiefly involved. In very few instances the lower end of the ileum may be affected the pathological process then extending upward from the caecum. The lesions may also sometimes extend from the caecum to the appendix. Any part of the large intestine may be affected but the ulcerations are somewhat more common about the caecum in fatal cases. Clark (1935) found in his series of 186 fatal cases the order of frequency of ulceration

instances they apparently pass directly through the basement membrane and muscularis into the submucosa. Here they are liable to migrate laterally and in depth for a considerable radius. In addition they often invade the capillaries and veins causing thrombosis of the blood vessels of the submucosa or muscularis with consequent necrosis of the tissues above. MacCallum (1906) has sometimes observed the amoebae underneath the endothelium of the branches of the portal vein as well as lying free in the lumen of such venules. The most typical amoebic ulcer is the flask shaped one due to the spreading out of the amoebae in the submucous coat the edges being formed of the overlying basement and mucous membranes Fig 123. The accumulations of amoebae in the submucosa tissues are attended by a low grade inflammatory reaction with oedema lymphocytic infiltration cytotoxicity and fixed tissue proliferation. The amoebae are particularly found in the oedematous tissues beyond the areas of most acute inflammation in the latter of which intestinal bacteria also play a part. In certain cases the tissues seem little able to resist infection and large gangrenous ulcers result the walls of which are soft and the bases of which are formed of blackish or greenish sloughing tissue in which numerous cocci bacilli and sometimes amoebae are found. These changes are certainly not produced entirely by the amoebae but are probably chiefly due to the bacteria. Another process sometimes observed in the intestine in amoebic dysentery is a diphtheritic one which is also influenced chiefly by the bacteria present in the intestine. In the healing of extensive lesions of the intestine the excessive formation of scar tissue sometimes leads to contractures and partial obstruction. The pathological lesions in the appendix liver and other parts of the body are especially discussed under Complications pp 525-540.



FIG 120—Amoebic dysentery. The glandular tissue of the intestine is invaded by the amoebae. The blood vessels are also invaded and the surrounding tissue is destroyed. (Curtis, Fish & Johnson)

**Distribution and Pathogenicity of the Amoebae in the Body**—In addition to inhabiting the large intestine amoebae have been repeatedly found in the neighboring tissues and abdominal cavity and in abscess of the liver lung pleura and brain.

was, the caecum 87.3 per cent, ascending colon 57.1 per cent, rectum 39.6 per cent, and appendix 33.3 per cent.

The most striking change at autopsy is the thickening of the wall of the large intestine with some vascular injection. The thickening is always more marked in the submucosa but may affect all of the coats. On opening the intestine the characteristic lesions consist chiefly of haemorrhagic catarrh of raised hemispherical areas of infiltration in the mucosa often surrounded by zones of hyperaemia or haemorrhage and of ulcers.

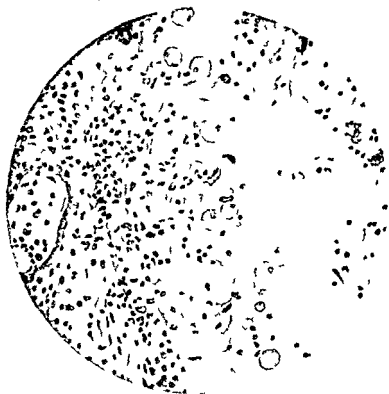


FIG. 125.—Edge of ulcer in the submucosa showing extensive cytolysis and numerous endamoebae ( $\times 170$ ).

tions. A very early lesion consists of small raised haemorrhagic areas which later lose their surface epithelium. The destructive lesions of the intestine consist of small erosions which may involve the mucosa alone or ulcers with a crater-like appearance with undermined margins and finally of large irregular shaped ulcers whose bases are formed of muscular coat or even peritoneum. In the latter case peritonitis with perforation is liable to occur. These large ulcers are often formed by the coalescing of smaller neighboring ulcers through sinus communications formed in the submucosa; the overlying muscularis and mucosa then sloughing off. The amoebae invade the glands of Lieberkuhn and pass into the crypts of the mucosa sometimes producing there small areas of necrosis. In other

reported as occurring in the head of the excised femur and in the immediate neighborhood of necrotic areas in the eburnated surface of the joint. Kolford, Swezy and Boyers (1921) also announced the discovery of *E. histolytica* in the glands in 7 cases of Hodgkin's disease, also in the bone marrow of these cases and in the intestine. These observations of Kolford and Swezy *et al.* have not been confirmed. They illustrate the similarity that may exist in the appearance of certain human cells to *E. histolytica*.

Craig (1940) points out that practically every organ and tissue of the human body has been reported as being invaded by *E. histolytica*, but that the vast majority of such reports have been based upon insufficient evidence that the cells so diagnosed were actually amoebae.

Thus Cherefeddin (1927) reported 5 cases of amoebic nephritis in which the urine always contained amoebae, red blood cells and casts, and Franchini (1928) 10 cases of amoebic cystitis. Other reports have been made of amoebic bronchitis without lung abscess.

In regard to these and other inflammatory manifestations which have been reported by different writers, such as amoebic bronchitis and pulmonary congestion, nephritis, cystitis and urethritis, Deschamps and Meinotte after 4 years' study in Morocco have concluded that the incontestable proof of the amoebic nature of such conditions has not been given.

The writer also feels that there is grave doubt of the amoebic origin of such conditions, and that the presence of other cells, frequently macrophages containing red blood corpuscles, have been confused with amoebae.

However, extensions of the infection with *E. histolytica* from lesions of the intestine and liver to the skin, sometimes results, producing extensive necrosis and sloughing of the skin. Such lesions of the skin have been reported in recent years by Engman and by Meleney (1937).

Ngai and Frazer (1933) in perianal condylomata, and Wu (1937) in China, have also found amoebae in sections of perianal ulcers, fistulae and warts, and Manson Bahr (1938) has found amoebae in a lesion of the skin due to an old colostomy wound. Wu (1938) has reported an unusual amoebic infection of a nasal pharyngeal polyp, the size of an orange, removed from the back of the nose.

### CLINICAL MANIFESTATIONS

The severity of the symptoms varies as greatly as does the extent of the ulceration. The onset is usually insidious.

**Incubation Period.**—It is frequently impossible to determine the natural period of incubation in amoebic dysentery because there is seldom accurate information as to just when infection occurred. In some individuals it apparently has been but a few days, but in others it extends over many weeks or months. In the epidemic in Chicago in 1933 the incubation period in some cases was short, the symptoms of dysentery appearing 8 to 10 days after exposure, while in other cases months elapsed before symptoms of dysentery appeared. The incubation period probably depends particularly on the susceptibility of the individual and the severity of the primary infection, and whether there are superficial erosions in the mucous membrane of the large bowel at the time of ingestion of the cysts. The course of the disease is usually protracted, marked by recurring periods of active dysentery alternating with periods of remission during which there may be troublesome constipation. The disease may

A number of authentic cases of the presence of amoebae in perineal sinuses and in the bladder and urine have also been reported. Often when present in the bladder and urine there has been a sinus connecting with the intestine. Amoebae have also been reported in ascitic fluid in the pelves and ureters of the kidney in amoebic nephritis, cystitis and urethritis, ovarian abscess and salpingitis, abscesses of the spleen, kidneys, and suprarenals in bronchitis, necrosis of the ribs or jaw bone in abscess of the mouth in parotitis and in pyorrhoea alveolaris. The species so commonly found in the mouth *Endamoeba gingivalis* has attracted particular attention through the reports in 1915 of Smith and Barrett and Bass and Johns which intimated that the organism was the



FIG. 127.—Nodular areas and ulcerations in the caecum

cause of pyorrhea alveolaris (Riggs' disease). While this view is generally no longer held, this species of amoeba which is often found in the mouths of children with normal gums is nevertheless frequently much more common in persons with pyorrhea and unhealthy gums. There is however no conclusive evidence that it is primarily pathogenic, though it would be equally wrong to conclude that it may not modify and even extend the lesions in certain necrotic processes. Many observers regard it as harmless.

Warthin (1922) has reported one case of the invasion of the testes and epididymis by *E. histolytica* in a patient suffering with chronic amoebic dysentery. The epididymis showed marked dilatation of the ducts and in these occurred masses of spermatozoa and amoebae. There was little evidence of an inflammatory nature.

Kofoed and Swezy and Ely, Reed and Wyckoff (1922) reported the discovery of *E. histolytica* in the lesions of a non-bacterial type of arthritis. The amoebae were

attention in the earlier stages of the disease are diarrhoea abdominal pain and later soreness upon pressure over the abdomen. There is usually no nausea and the appetite may remain unaffected. Only when the disease progresses still further does the diarrhoea or mucus and blood in the stools become a prominent feature. It is on account of this fact that the term amoebic dysentery is not applicable to all cases of the malady and that the term amoebic enteritis or amoebiasis is a more appropriate term for the infection.

**Forms with Acute Onset**—The onset of amoebic dysentery as observed by the physician in over one half of the cases is acute. Abrupt onset may occur from the formation throughout the large intestine but particularly in its lower portion of very numerous small and superficial ulcers or from secondary infection with streptococci or *Bacillus dysenteriae*.

Cases with diphtheritic or gangrenous lesions may be classified clinically under this division and in the latter instance portions of sloughing tissue may be passed in the stools. Headache nausea and chills may usher in the attack. Soon afterwards spasms or gripping pain are felt in the abdomen followed by frequent loose movements. At first the pain is intermittent and acute being most severe in the umbilical region but later it usually becomes dull and continuous and is then referred to the sigmoid area. There may be from 15 to 50 or more bloody mucous movements in 4 hours. A high leucocytosis is frequently present. Colicky pains in the abdomen with rectal and vesical tenesmus may occur and fever and vomiting appear. Great exhaustion sets in the heart action becomes feeble and death results from cardiac failure and collapse or the condition may temporarily improve and gradually assume the chronic form. It is in the acute form that wild delirium may be observed before death.

**Advanced and Chronic Forms**—Patients with the advanced and chronic forms of the disease usually suffer with recurrent attacks of diarrhoea in which from time to time blood or mucus is passed with some pain. Between the attacks of diarrhoea the stools may be formed though they also frequently contain some mucus and blood. The number of stools may vary from 2 or 3 in the morning to 10 or 15 or more during the day. There is frequently a dull aching pain in the abdomen or in the back and at times a sudden and intense desire to defecate. The general health finally suffers and emaciation begins to be evident. The patient often appears sallow and then becomes anaemic and sometimes emaciated the skin becomes dry and dull yellow in color and the muscles soft.

**The Blood**—Hinman and Kampmeier (1937) who studied the blood of 89 cases in New Orleans found the red blood corpuscles were below 4 500 000 in 64 per cent or 57 cases and less than 4 000 000 in 39 of these. Only 9 were below 3 million. In 76 cases 3 or 42 per cent showed less than 70 per cent of haemoglobin (Talquist). Craig (1934) points out that in chronic cases of long standing the red cell count may fall to 3 000 000 or rarely even to 500 000. Gastric analyses were made by Hinman in 4 cases 5 showed definite hypochlorhydria and 3 achlorhydria (included in the 25).

These periods of low fever accompanied by leucocytosis may occur from time to time. The leucocyte count is often below 10 000. A leucocyte count of 15 000 often



last 30 or 40 years. There may be merely a mild diarrhoea, or the dysentery may be severe with 12 or more bowel movements daily, accompanied by marked colicky pain and tenesmus. Rarely the onset may be fulminant and death may occur within a week. As a rule the onset is less abrupt and the symptoms less acute than in bacillary dysentery. The disease may be symptomless, or be associated with vague abdominal pains, digestive discomforts or other complaints which do not suggest intestinal disease. These mild or symptomless cases have been shown to outnumber greatly the cases with clinical dysentery. They have been called 'carriers' or 'cyst passers' in which the lesions produced may be well marked or insignificant. In such individuals (if untreated) the carrier state may last for long periods. A carrier may develop clinical symptoms at any time if his resistance is lowered, but most of them never do so. The reason for such marked variations in the reaction of different individuals to the infection is not entirely understood. Some of the influencing factors have been discussed on page 485. Since the symptoms of the disease differ so greatly in character and severity, in order to discuss the clinical course the cases may be grouped conveniently under (1) mild or latent forms (2) those with acute onset (3) advanced or chronic forms.

It should however be understood that this division is purely arbitrary. Cases with grave intestinal lesions may sometimes come to autopsy in which the individuals had during life no intestinal symptoms sufficiently prominent to attract attention. While individual cases of amoebic enteritis may vary widely there are nevertheless some features which are common in at least the majority. These are the irregular course marked by periods of intermission and exacerbation, abdominal symptoms, the appearance of mucus in the stools and the tendency to chronicity. A phenomenon peculiar to the malady is the occurrence of amoebic liver abscess.

**Mild or Latent Forms**—The onset is usually insidious and a great many of these infections may remain undiscovered for a considerable period of time. Frequently the patients are not able to tell when they began to realize they were not in good health. There may be complaint of some lassitude, abdominal discomfort or dyspepsia. Slight intestinal disturbances consisting of moderate diarrhoea or constipation may appear. Occasionally the abdominal pains become severer or there may be an outbreak of diarrhoea which causes the physician to examine the stools when amoebae, sometimes mucus and even red blood corpuscles may be found. Cases may never advance beyond this latent stage. Either under treatment or even without it the patient may overcome the infection and the parasites disappear from the stools or the patient may remain a carrier for years passing amoebae and cysts in the dejecta. In by far the greater number however if pathogenic amoebae are present the symptoms sooner or later increase in severity and in the event of recovery not taking place the disease passes to a more advanced stage. Such cases may then be more properly classified either as those with acute onset or advanced or chronic ones. The symptoms most likely to attract

the temperature ranged between 101-104 F in all but 3 33 had a relative as well as absolute polymucleosis 23 between 76 and 85 per cent and 9 over 85 per cent

In the chronic forms indigestion and flatulence often develop The temperature may be subnormal in the morning slightly elevated in the afternoon Moderate albuminuria may occur and a few hyaline casts appear in the urine As the disease progresses there is a marked loss of appetite the emaciation may become extreme the abdomen sunken bed sores appear and death follows from exhaustion or terminal infection

Another type of the chronic form is that in which there is nothing more than an intermittent diarrhoea often alternating with constipation and accompanied usually by slow but gradual loss of flesh If the severer intestinal lesions in which the destruction of tissue has been marked subsequently heal extensive cicatrices are apt to form sometimes with resulting narrowing of the bowel The chronic catarrhal condition may also persist

**Course and Prognosis**—The course of the disease is very variable and is not self limited The increased employment of emetin and other amoebicidal drugs in the treatment of amoebiasis has often greatly modified its clinical aspects In the United States one sees fewer of the more severe and more advanced cases of amoebic dysentery than formerly and fewer serious complications In many cases which would formerly have been considered hopeless the patients now often recover In untreated patients who are exposed to hardships in the field and away from physicians and hospital facilities the death rate may be as high as 30 to 40 per cent while in cases treated properly with emetin or other suitable drugs the mortality should not be over 10 per cent In the uncomplicated cases those with acute onset including the gangrenous forms usually have the gravest outlook Hiccough which is seen particularly in the severer forms often indicating involvement of the peritoneum and the approach of exhaustion and death is a very unfavorable sign Death may occur from the gravity of the intestinal lesions from exhaustion in protracted cases from severe complications particularly abscess of the liver from a terminal infection from intercurrent diseases or from severe intestinal haemorrhage The severity of the intestinal lesions and abscess of the liver are the most frequent causes of death

### DIAGNOSIS

The diagnosis of amoebic dysentery can often be made with certainty only in the laboratory as there are other forms of dysentery which it may be impossible clinically to distinguish from it and one who attempts to form a diagnosis from the clinical manifestations alone will make frequent mistakes

The physical examination of the patient with moderate symptoms reveals little except sometimes abdominal spasm and tenderness over the affected portions of the bowel The temperature may be normal or slightly elevated especially in the afternoon The pulse may be only slightly increased in rate and unaffected in volume and tension

suggests some complication especially liver abscess Solarino (1939) found the blood practically normal except that the leucocytes ranged from 5 000-15 000 the higher counts usually in the earlier acute stages Hinman and Kampmeier (1937) performed



FIG 128—Colon in amoebic dysentery showing advanced necrotic sloughing lesions with filamentous forms projecting into the lumen of the bowel

differential counts of 105 patients 73 or 69.5 per cent had a polymorphonuclear ratio of 75 per cent or less and 59 or 56.3 per cent between 56 and 75 per cent Thirty-one had a leukocytosis over 10 000 and 11 of these over 15 000 Of the 11 liver abscesses was proved in 3 and was probably present in 2 more With counts of 15 000 or over

show cytolysis and consist of scanty ragged cytoplasm surrounding pyknotic nuclei

Callender (1934) and others emphasize that in bacillary dysentery the exudate in the stools contains very large numbers of pus cells some 90 per cent being degenerated leucocytes while large macrophages are present which may contain red blood corpuscles. The last have sometimes been mistaken for amoebae but do not possess the motion of the



FIG. 30.—Stool in bacillary dysentery showing amoebae (A) and some of which show tendency to clump. Charcot Leyden crystals present (Army Medical Museum No. 3818)

trophozoites of amoebae. On the contrary the cellular exudate in the stools of amoebic dysentery usually contains few or no pus cells and there are epithelial and other tissue cells swollen and degenerated leucocytes Charcot Leyden crystals also may be present. Thompson and Robertson believe the presence of Charcot Leyden crystals in amoebic dysentery is a valuable diagnostic point. However Stitt and Clough (1938) state that while they are suggestive they are not pathognomonic of amoebic dysentery. Manson Bahr has found these crystals in the stools in malignant disease of the rectum in mucous colitis and in helminthic and coccidial infections.

Other disease conditions that may be especially mistaken for amoebic dysentery are bacillary dysentery, mucous colitis, chronic enteritis and schistosomal and balantidial dysentery.

Stitt calls attention to the fact that it is possible to differentiate bacillary from amoebic dysentery by the more sudden and acute onset of the former together with fever and other evidences of toxemia, also the pulse rate is somewhat more rapid in bacillary than in amoebic



FIG 129.—Stool in bacillary dysentery (early stage) showing (A) malarial parasites containing light bodies surrounded by a halo—red blood cells and dark bodies which are probably nuclear detritus. Polymorphonuclear leucocytes are numerous and many show a ringing of the nucleus resulting from a toxic degeneration. (Army Medical Museum No 39105) Compare with Fig 130

dysentery and the number of stools is usually greater although the amount of each stool is less in quantity.

In amoebic dysentery the stools are often fluid, relatively copious and contain faecal material and varying amounts of fresh and altered blood which may give a dark brownish or reddish color and there may be much blood streaked or brownish mucus. This contrasts with the scanty, watery, non-faecal passages containing masses of whitish mucus flecked with bright red blood in the bacillary type of dysentery. Microscopically the amoebic stools show mucus and numerous red cells, often clumped and degenerated and very few pus cells or phagocytic cells which are numerous in the bacillary type. The cells which are present mostly



E d m b hu t lyt		E d m ba		E d m x		l d m ba b t chl		D lama b f gel			
20-30 ( 5-60 )		0-30 ( 0 15-5 )		6-1 ( or 6-5 )		9-13 ( 18 0 )		5-12w			
Ch c r n t p hly mov d f m h t d p ly st n sh g t t y fl w n l m t t ght f c s f l d So b om s l a t e p s b g t a f w l g d pod blad h p l p hly l whch ep l p hly l t g composite t lyol t pla m Th m f m t m y o t m b hou d s up nd d		F hly m d m y sh w rule h w a t vity a l g r h th l t l l m o m t r y m v m t Mo t s c a t ch dly n h g r l sh p w t h o ev d t p o g n p m t o o f l g e f r blad l k pseudop d a a ly se D g r t e m t o n l o d d f r m a f a t l y o d d f r m a b l f o m m m l r l m s c l L h l d l		A r u l t y c t v H a s s l o w p r g e t e m o n n t f sh p p a t s L a t r h w a m m t v h g e o f sh p e I s e u d p o d s f w b l t a d t h c k S o n o u n d p a d d a a d t h m o l e s d g t a f r m a t h e m o t c m m o l y s e e n n a t l		G a l l y b t s l g hly m o t i f e M o e m n t s m l t t E o d Q t l y r s e m b l a s w h n l e s m l l s p e m e s c l E c o l l Q k l y d g r a t s d		D r l y s l w p e c u l r t s E d o p l a s m f l y g r n u l r n d b m g n o i n p p e a r c U e l l y c o n t a n s n m r u s f o o d v e u l a c h a r g d w o t h m l b a t e r n R d b l o d l l t h n g t d C y t e d v i l y d e s c r i b e e p d c r y s t		A t e m t i l y p u d p o d h y l b l t d l e f l i k e	
End pl m c l l n ly g l a r d u l m a p p c e M v c o t a m d b l o o d c l l d f g m a t (h o s t t e l l s b u t b c t r n d t h p r t i c l n h t e f e s p b l y v g e t e d r m l y		E d pl m b l e y g r l a n o d u a l l y o t a u m a s d v a u h a g d w t h b t m y a t v g e t b l d b r n f n d o t h r p r t l a d e r v e d i m h o t f c a R d b l o d c l l n o t u s l y g t d O t h v a c l p d l h a p e d c t a g f d d e n n o h a r p l f d m t t n a r p a t t h t p l a m f o m t h e d p l m		Q p l y s f w t e w r i b y f e t u a L a n d p l a m f i n d l y m t u t e f d v c u o l s o n t a g g s t d b a c t r i a R e d B l o o d l l a t l		U t d a l l y n e v b l S t i d m m o l y b u n l t M a t l y u m b d l t h t N o b m a t n d t s i d r o p s o m l g b r o m t m g r l f t t l y r r g d t h		U t d a l l y n e v b l S t i d m m o l y b u n l t M a t l y u m b d l t h t N o b m a t n d t s i d r o p s o m l g b r o m t m g r l f t t l y r r g d t h			
4-7 (U t d d l t v l e S t p c u s f l e S t d h o w f l e S t d h o w b d d n g r u l K r y s o m m l p h m m t p r s		4-7 (U t d d l t v l e S t p c u s f l e S t d h o w f l e S t d h o w b d d n g r u l K r y s o m m l p h m m t p r s		4-7 (U t d d l t v l e S t p c u s f l e S t d h o w f l e S t d h o w b d d n g r u l K r y s o m m l p h m m t p r s		4-7 (U t d d l t v l e S t p c u s f l e S t d h o w f l e S t d h o w b d d n g r u l K r y s o m m l p h m m t p r s		4-7 (U t d d l t v l e S t p c u s f l e S t d h o w f l e S t d h o w b d d n g r u l K r y s o m m l p h m m t p r s			

Th 5-6 fth ma b 2 em b d f t p m el g d d p d m e u t f h

Wenyon and O Connor believed *Endamoeba coli* did not ingest red blood corpuscles and that if amoebae were found englobing red blood corpuscles they were certainly *Endamoeba histolyticae*. York and Macfie 1919 have shown however that non pathogenic amoebae of the Limax type will ingest red blood corpuscles and they therefore believe that *Endamoeba coli* may also ingest red cells.

Tyzzar and Guyman (1939) have observed a human case in which there was a polyp of the sigmoid and a blood streaked mucous discharge which contained an amoeba which frequently included red blood cells. A careful study of it including cultures and inoculation of kittens convinced them that the species was *Endamoeba coli* which had actively ingested the red blood corpuscles in the intestine.

It must be remembered that the characteristic features of the trophozoites and cysts of the various species of intestinal amoebae enumerated can not be made out in every individual parasite but that in every faecal specimen there are atypical forms particularly of the trophozoites which can not be positively identified. Several individual organisms should always be inspected.

Amoebae must also be distinguished from large mononuclear phagocytic cells such as those common in the stools in bacillary dysentery. The latter have larger more conspicuous nuclei than *E. histolytica* and often contain bacteria or vacuoles and even red blood cells. It is essential for diagnosis to demonstrate the typical motility of the parasite. This together with the presence of ingested red cells practically suffices to identify an organism as *E. histolytica*. If there is doubt as to the identification or if permanent preparations are desired films must be fixed and stained by the iron-haematoxylin method and the nuclear structure studied. This procedure should supplement not replace the examination of fresh preparations. As due from the time required for staining it is more difficult to detect amoebae in stained films than in fresh preparations.

During the first few days after the onset of the initial attack the organisms may be absent or sparse. At later periods they can usually be found. They may be absent or sparse in one specimen and abundant in the next but examination of properly selected material from 3 suitable specimens will reveal them in nearly all cases.

The distribution of chromatin in the nuclei is frequently an aid in differentiation. The examination of the cysts in stained preparations however usually gives more accurate results in the determination of the species.

For fixing and staining preparations Schaudinn's well known alcoholic sublimate (equal parts HgCl<sub>2</sub> and 95 per cent alcohol) and staining with iron-haematoxylin method has frequently been employed. Stutt Clough and Clough (1938) recommended Carnoy's fixative which consists of absolute alcohol 6 parts chl. reform 3 parts glacial acetic acid 1 part as excellent for any staining method. It is especially useful when followed by haematoxylin. Used in the cold it insures quick killing and fixation and fidelity of tissue elements when followed by haematoxylin stains. Immerse moist smear in fixative for 10-15 minutes and then in distilled water 10-15 minutes. Stain.

Donaldson's iodine-eosin stain as modified by Kofoid gives most satisfactory results. It should be freshly prepared as follows.

Saturated solution of eosin in normal salt solution 2 parts 5 per cent potassium iodide in normal salt solution saturated with iodine one part normal salt solution 2 parts. The smear is prepared for microscopic examination by rubbing out a minute bit of the faeces by rolling it on a round applicator stick in a small drop of normal salt solution and then in an adjacent drop of iodine-eosin stain. A single cover is placed on both drops and the smear is ready for immediate examination. Living flagellates and



Manson Bahr (1936) believes sigmoidoscopic examinations sometimes afford valuable information in diagnosis when ulcers are present in the rectal canal. Hinman and Kampmeier (1937) made proctoscopic examination in 299 cases and ulcers were seen in 261 or 87.3 per cent, and active amoebae in 252 or 84.3 per cent. The diagnosis should be confirmed in every case by finding amoebae in the scrapings from such ulcers.

**Laboratory Diagnosis**—The dejecta should always be carefully searched for amoebae. Demonstration of the motile trophozoites or cysts in the stools is essential for a positive diagnosis. The examination of the stools should be made as soon as possible after they are passed and the specimens should be collected free from urine, as the amoebae often die and disintegrate in the stools a short time after they are passed. The amoebae should be found living and motile. In this condition they are not likely to be mistaken for phagocytic cells. A fresh warm stool is most favorable for examination and if it contains particles of mucus these should be specially examined.

Stitt has obtained beautiful results with vital staining by tinging the suspensions with 1 per cent aqueous solution of neutral red. Examine with the low power ( $3\frac{1}{2}$  inch (AA) objective  $6\times$  ocular), and use the high power for identification only. Magath 1935 has emphasized the great practical importance of this point. If the stool is formed motile organisms can rarely be found in the faecal mass but may be demonstrated in mucus adhering to it. In such cases one may give a saline purge and examine flecks of mucus contained in the first fluid stool. It has been generally recognized that the chance of finding protozoa is much increased by such catharsis. Oil (and barium) must be avoided. Hegner and his associates point out that in Mexico 25 per cent of the diagnoses for *E. histolytica* and 21 per cent for *E. coli* were based on trophozoites found in purged stools.

If there are ulcers in the rectum the organisms can usually be demonstrated easily in scrapings from the ulcers obtained through a proctoscope. They can often be obtained more simply by passing a rectal tube as deeply as possible into the rectum and examining the fleck of mucus caught in the eye of the tube. The organisms are not evenly distributed in the stool and several particles should be examined.

After the presence of the motile living forms has been determined the species should be differentiated. It is often difficult to distinguish with certainty the species of intestinal amoebae from an examination of the living motile stages.

The two species of *Endamoeba* which most commonly occur *histolytica* and *coli*, are not strikingly different in their appearance in the vegetative stage. They measure approximately 20–30 $\mu$  in diameter rarely 40–50 $\mu$ . The nucleus of each is small in proportion to the size of the cell very poor in chromatin which however is coarser in *coli*. In *E. histolytica* the ectosarc is often strikingly clear and hyaline and phagocytosis is active the amoeba frequently containing red blood corpuscles with usually few if any bacteria. *Endamoeba coli* usually contains no red blood corpuscles but much more frequently ingests bacteria.

The cysts of *Endamoeba histolytica* measure from 5 to 20  $\mu$  according to the strain or race. The cysts of a given strain are usually uniform in size. They are spherical or oval and the cyst wall is colorless and perfectly smooth and formed of a single layer. The cyst when first formed is uninucleate. The cysts are passed in the faeces in the uninucleate binucleate or quadrinucleate stage. Kuenen, Swellengrebel and Scherf have found cysts of *Endamoeba histolytica* containing 3, 6 and even 8 nuclei. The zoological diagnosis of the species hence at times is further complicated particularly since *Endamoeba maxima* may sometimes though infrequently also show 8 nuclear cysts. However, Craig (1937) points out that 8 nuclear cysts in *E. histolytica* are very rare and practically not encountered in fresh stools. The cysts of *Endamoeba coli* measure from 10 to 30  $\mu$  or even more. The cysts with 2, 4 or 8 nuclei may occur in the stools. If mature eight nuclei are usually present. In fact about 80 per cent of the cysts of this or not found in the stools are said to be 8 nucleate. Occasionally however supernucleate forms with 16 nuclei may be seen. The forms with only 4 or 7 nuclei. The mature cysts of *E. histolytica* are typically also measure usually from 6 to 10 in length and 5 to 8  $\mu$  in width. Kolod and his associates believe that the structure of the nucleus of *Endamoeba maxima* when stained is an absolute diagnostic criterion of this species and they agree in this opinion with Wenyon. In *Endamoeba histolytica* as noted there is a central karyosome and the peripheral chromatin is scattered around the nuclear membrane in granules of a small size whereas in *P. nana* chromatin is massed in a single large clump and there is absence of chromatin granules on the nuclear membrane.

In *Endamoeba butschlii* the cysts measure from 5-10. The cytoplasm is vacuolated and a large glycogen vacuole is usually present. No cysts have been demonstrated in *Dientamoeba fragilis*.

The cysts of the most common species given above must also be distinguished from the cysts of the amoebae of the *Lima* type which are found in stale stools are always small uninucleated and often have a thick wall of brownish color.

Cysts of *Chilodactylus mesilis* are often lemon shaped and are characterized by the presence of tubules of the cystosome beside the nucleus.

Cysts of *Giardia intestinalis* are easily recognized by the egg shaped appearance they are about 8-12  $\mu$  in length (commonly oval) and with a can be seen the characteristic pair of curved deeply staining bodies interpreted as parabasal bodies usually 4 apical nuclei are clustered at one end together with the flagella.

*Blastocystis hominis* is not an uncommon inhabitant of the human intestine. It has sometimes been mistaken for cysts of forms of *E. histolytica*. It is spherical or oval in shape and may measure from 5-40  $\mu$  though usually 10-15  $\mu$ . It has a more delicate capsule than the cyst wall of *Endamoeba* and contains a very large vacuole which reduces the cytoplasm to a narrow rim. The oval narrow layer is differentiated and contains refractive granules and one or more refractive nuclei. Its differentiation from the cysts of amoebae is still more striking in stained preparations.

The differentiation of the pathogenic species of amoebae may in addition be completed by animal inoculation as described on p. 488.

**Differential Diagnosis**—It should be borne in mind that infection with other pathogenic organisms may coexist particularly in cases with acute onset or acute symptoms. *B. dysenteriae* should be sought for in plate cultures made from the stools on litmus lactose agar. The agglutinative and bacteriolytic reaction of the patient's blood may also be tested with *B. dysenteriae* but this frequently fails to give definite information. Dysenteric symptoms due to *Balanitium coli* or schistosomal infection of the intestine should also be excluded by microscopical examination of the faeces for the presence of the ciliate or ova of *Schistosoma*.

unstained cysts appear in the unstained part. In the stained area the bacteria faecal particles and the intestinal yeasts (except the larger forms) stain at once. Against the pink background the protozoan cysts stand out clearly as bright spherules which soon become tinged with the iodine to varying tones of yellow while their glycogen filled vacuoles when present turn light or dark brown according to their mass. The nuclei become more clearly defined as the iodine penetrates especially in *Endamoeba coli* and *Endamoeba histolytica*. They are detected with difficulty in this stain in *Endolimax nana*.

The cysts should be looked for in solid or semisolid faeces. Practically identification of *E. histolytica* depends upon distinguishing cysts with the 4 nuclei. This is difficult in unfixed preparations and often impossible unless the preparation is mixed with dilute iodine solution or iodine eosin solution. Chromidial bodies should not be mistaken for nuclei. In the case of strains which form small cysts (5-7 $\mu$ ) differentiation from *Endolimax* may be difficult and require fixed stained films. They must also be differentiated from *Blastocystis*.

Cysts are unevenly distributed in faeces and are often sparse. One should examine material from several particles or prepare a homogeneous suspension from a considerable portion of the stool. Dobell concluded that examination of a single stool would reveal cysts in only a third of infected cases and to exclude infection with reasonable certainty 6 examinations are required. Svensson and Lunders demand 10 examinations. The concentration method is of assistance. Care should be taken on the part of the examiner to avoid infecting himself.

Concentration of Cysts—Faust (1939) and his associates have found a most satisfactory method of concentrating both cysts of protozoa and ova of worms by making up a 1-5 suspension of faeces in physiological salt solution straining through cheesecloth or wire gauze and then centrifuging 2 cc. in a Wassermann tube with water added (45 seconds at 1640 R.P.M.). The supernatant fluid is then poured off, zinc sulphate of specific gravity 1.180 (331 grams of U.S.P. granular zinc sulphate in a liter of distilled water) added, the sediment stirred up and the tube centrifuged again. The surface film is then removed by means of a 5 mm. wire loop or enough more zinc sulphate is carefully added to form a meniscus to which the surface of a clean slide is touched. The number of positives was nearly twice as great as with simple smears in Faust's series.

Differentiation of Cysts—In the encysted stage in *E. coli* the nucleus with its coarser chromatin and eccentric karyosome is the chief figure distinguishing it from *E. histolytica*. The mature cyst of *E. coli* contains usually 8 nuclei but before excystation it may lose some of its nuclei to the number of 1 to 4 containing only 4 to 7 instead of the typical 8. Also supernucleate cysts may occur. Brooke (1940) in the study of a case found the average number of nuclei per supernucleate cyst to be approximately 15.

In *E. histolytica* the chromatin of the nucleus consists of a small central granule (endosome) with many small granules on the nuclear membrane while in *E. coli* there is a larger central granule (endosome) of chromatin which is usually eccentric and there are many larger granules around the nuclear membrane. In *Iodamoeba butschli* the chromatin of the nucleus is largely concentrated in a central karyosome and there is absence of chromatin granules encrusting the nuclear membrane. In *Diendamoeba fragilis* there are no cysts known. Frequently 2 nuclei are present in the trophozoites.

difficulties in obtaining a satisfactory antigen. The serums of 90 patients at either the Mayo Clinic or the Vanderbilt University Hospital all harboring *Endamoeba histolytica* were tested for complement fixation with a variety of amoebic antigens. The serums were examined independently in the laboratories of the 2 institutions. There was general agreement in the findings of the two laboratories but owing to the use of multiple antigens one laboratory obtained more positive reactions than the other. Twenty nine of the 90 cases or 32 per cent gave positive complement fixation reactions.

While a few cases of acute amoebiasis gave a higher percentage of positive results there were cases in which issue was demonstrated to have been invaded by *E. histolytica* in which the reactions were negative. It seems obvious that considerable improvement in the preparation of antigen will be necessary before the test can be considered of great value in diagnosis and the value of the reaction ascertained.

### TREATMENT OF AMOEBIC DYSENTERY

*Emetine hydrochloride* ( $C_{22}H_{40}O_4N_{12}HCl$ ) is the most effective drug in the treatment of acute amoebic dysentery and a number of clinicians with a wide experience still regard it as the standard drug for treatment (Strong 1921 Brown 1935 deLangen and Lichtenstein 1936 and Sellards 1937). DeLangen and Lichtenstein write that in spite of certain dangers and the fact that in a large number of cases emetine has failed to cure and to prevent relapses nevertheless this drug remains the greatest contribution towards the treatment of amoebiasis that we have as in countless instances it cures the amoebiasis quickly and thoroughly and in almost as many instances it stifles each relapse completely. In amoebic liver the drug works wonders. It should be given during the acute stages of the disease to an adult in doses of 1 grain (0.065 gram) emetine hydrochloride in 1 cc of distilled water subcutaneously or intramuscularly once a day the drug being continued for a week or 10 days. Children should receive proportionately smaller doses not to exceed 1 milligram (0.015 grain) per kilo of body weight per day. Treatment with this drug undoubtedly has saved many lives. The patient however must be carefully watched for toxic symptoms and the margin between the therapeutic dose and the toxic dose is small. Sellards cautions that the blood pressure should be taken before the administration of emetine is commenced as the earlier sign of the toxic action of emetine is a fall of blood pressure and irregularity in cardiac action. Mackie (1937) emphasizes that it is contraindicated in advanced myocardial disease and that it should be used with great caution if at all in the presence of organic heart disease. The toxicity of emetine must be emphasized. The physician should bear in mind not only that the drug is poisonous but that it is excreted slowly by the intestine and kidneys and that its action may be cumulative. Dale (1917) demonstrated experimentally that repeated doses of emetine produce cumulative poisoning in cats and rabbits and Rosen Martin David and Leake (1935) have confirmed this fact by experiments on guineapigs. Kilgore emphasized

**Other Means of Diagnosis**—In cases in which amoebae are present in small numbers in conjunction with the microscopic examination of the faeces attempts may be made to secure cultivation of the amoebae from freshly passed stools by the method described under "Cultivation" page 483 St John, Cleveland and others, have emphasized the value of this method of diagnosis. However McGrath and DeYoung (1936) have pointed out that the results obtained by this method may at times be misleading. Free living amoebae accidentally ingested with the food may also grow in such cultures. Further experience is necessary to determine the practical value of the method.

**Complement Fixation Test.**—Craig has reported upon the use of the complement fixation test in diagnosis in which the serum of the patient is tested with an alcoholic extract of cultures of *E. histolytica* as an antigen. A number of modifications of the test have been made. In the earlier experiments since in the cultures of the amoebae as well as in the material from the intestine containing the amoebae large numbers of bacteria were always present, it seemed possible that their presence might complicate the interpretation of the reaction.

Stone (1935) has prepared a practically bacteria free antigen by extracting with alcohol the washed cysts. Craig reports that he has used this complement fixation test for 8 years. Of 1500 individuals tested 17.5 per cent gave a positive reaction and in 89 per cent of these cases *E. histolytica* was demonstrated in the faeces. Craig (1937) states that the complement fixation test should not be employed except as a check upon the results when it is possible to have the stools examined for the amoebae. He points out that the reaction becomes negative after the elimination of the infection.

Yamamoto (1936) has also demonstrated the existence of specific complement fixing substances in the sera of both acute and carrier cases of amoebic infection. The most definite results were obtained by the use of alcoholic and 20 per cent cholesterinized alcoholic extracts of the amoebae. He states the antibodies gradually disappeared from the blood as cure takes place.

Chiraogawa (1936) by the use of an alcoholic extract of amoebae in cultures has demonstrated immune bodies in 23 cases of amoebic infection. Spector (1936) found that the serum of persons harboring *E. histolytica* which produce small cysts failed to give positive complement fixation reactions with an antigen prepared from a large race whereas serum from persons harboring the large race gave a positive reaction in almost every instance. However Frye and Meleney (1938) found that 5 out of 14 persons harboring a small race of *E. histolytica* gave a positive complement fixation reaction for amoebiasis the antigen being prepared from a strain of the large race.

Paulson and Andrews (1938) investigated the complement fixation test in 150 cases which had been studied clinically and parasitologically. These sera were submitted to one or more laboratories where the amoeba complement fixation test was being carried out with one or more antigens. Positive results occurred more frequently amongst those individuals in whom amoebic infection had been determined microscopically than in others but the numerous falsely positive results showed that the test may be unreliable in an individual case. It is hence only a diagnostic aid.

Meleney and Magath (1940) have made a further careful study of the value of this test in the diagnosis of amoebiasis. They emphasize the

difficulties in obtaining a satisfactory antigen. The serums of 90 patients at either the Mayo Clinic or the Vanderbilt University Hospital all harboring *Endamoeba histolytica* were tested for complement fixation with a variety of amoebic antigens. The serums were examined independently in the laboratories of the 2 institutions. There was general agreement in the findings of the two laboratories but owing to the use of multiple antigens one laboratory obtained more positive reactions than the other. Twenty nine of the 90 cases or 32 per cent gave positive complement fixation reactions.

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severe cases of peripheral neuritis after treatment with emetine and reported 10 such cases

The trouble generally manifested itself in general muscular pain and weakness especially in the legs going on sometimes to paresis. Wrist and toe drop were common. The symptoms disappeared gradually on stopping emetine. Belazco Spehl and Collard Balfour and Lyman Levy and Rowntree and Johnson and Murphy have all reported cases of poisoning due to emetine. Johnson and Murphy had 2 deaths and 5 other cases of poisoning which they believed were due to the drug. The fatal cases had received in all 23½ and 25 grains of emetine each in divided doses. Levy and Rowntree also report in detail 2 cases of poisoning first in a case of chronic amoebic dysentery the patient received 29 grains in 20 days and suffered from diarrhoea, muscular weakness, acute renal insufficiency followed by death. The other an anaemic woman with pyorrhea alveolaris received 2 grains spread over 4 days and recovered after diarrhoea with blood and pus in the stools and toxic delirium. They have collected 20 cases of poisoning from the literature which they have tabulated. In 6 of these less than 10 grains were given. All recovered except the first case. The symptoms included diarrhoea with blood 4 cases, diarrhoea 3 cases, peripheral neuritis 5 cases, muscular paralysis 1 case, muscular weakness 1 case, purpuric eruption 1 case and toxic delirium 1 case. They point out that patients may differ markedly in their susceptibility to the drug and that the various commercial preparations vary widely in toxicity.

Shattuck has also emphasized the danger of poisoning from emetine and notes 2 other deaths from the use of this drug—one reported by Soca in 1912 and one by Bais in 1923.

Intravenous injection should not be employed.

Pogers considers that 15 grains of emetine is a fatal dose for an adult man. Hesse attributes the chief danger in emetine to contamination with strongly toxic cephalin. He refers to the profoundly irritative local action of preparations of emetine and to the depressing action on the heart and circulation after repeated small hypodermic doses in animals. Deaths in emetine poisoning occurring from cardiac failure and the irritating effect on the gastro intestinal tract. Levy and Rowntree have shown by electrocardiographic studies that the cardiac irregularity produced in emetine poisoning is due to fibrillation of the ventricles from which the animals may recover. Berman and Lenke have shown by experiments on rabbits who were given lethal doses that ventricular fibrillation occurs. Epstein has also shown that it may act directly on the myocardium and may produce myocardial necrosis and degeneration. Sayid (1933) also reports 2 cases which developed severe auricular fibrillation after the use of emetine—one previously had mitral regurgitation but in the other the heart was apparently healthy. Both recovered. The consensus of opinion appears to be that emetine acts as an emetic but this effect follows only upon administration by the mouth. In the treatment of amoebic dysentery it should be borne in mind that the diarrhoea produced by large or prolonged doses of emetine may be confused with that produced by the dysenteric process.

All cases however do not by any means yield to treatment with emetine and relapses after the use of the drug in the doses advised in this article are not very uncommon. Its curative action often stands in direct proportion to its employment early in the acute attack. In cases with advanced lesions where there is much destruction of tissue and where secondary infection of the lesions with intestinal bacteria has occurred, its good effects are not so noticeable. For emetine cannot cure such lesions. It can however, destroy the amoebae responsible for the lesions.

Craig (1937) believes that emetine should be used only for the purpose of controlling diarrhoea or dysenteric symptoms for which purpose he

states it is unexcelled. He however has found this drug often unsatisfactory for the elimination of the cysts and treatment of carriers in more chronic cases. In the treatment of 130 patients in which at least one third were given 2 courses of treatment with emetine in 81 per cent of the cases in which the amoebae were destroyed or disappear from the stools by treatment cysts were later found in the stools within 40 days after cessation of treatment.

On account of the toxicity of the drug and its cumulative action if a second course of treatment with emetine is required to eliminate dysenteric symptoms it is advisable to give a rest period of 10 days to 2 weeks before repeating the course of treatment.

*Emetine Bismuth Iodide*—Manson Bahr (1936) believes that the primary course of emetine should be supplemented by the double iodide of emetine and bismuth containing 26 per cent of the emetine alkaloid. It is best given in hard gelatin capsules. The maximum individual dose for an adult is one 3 grain (0.2 gram) capsule by the mouth given at bed time for 10 to 12 consecutive days. Children and women should be given proportionately smaller doses. The drug is an insoluble powder from which emetine is set free by contact with the intestinal juices. Notwithstanding its extreme slight solubility in the gastric contents some nausea and even vomiting may follow its administration hence it is advisable that the patient should be in bed and that only a very light diet be partaken of a few hours before administration. The drug is useful especially in persistent passers of *E. histolytica* cysts. The writer has often prescribed it for travellers upon expeditions in the field. The drug has been particularly recommended by Dale Low, Castellani and many others. Manson Bahr has found that in many cases the full course of 30 to 36 grains is not necessary or advisable and that it is often not necessary to administer more than 19 grains altogether. In giving this drug the same precautions must be exercised as in giving emetine hypodermically. More recently (1941) in chronic cases he advocates that the dose should be reduced to 2 grains and should be given at 10 P.M. preceded by one grain of phenobarbitone nightly for 10 nights and that this treatment may well be combined with 7 oz. rectal injections of 2% quinoxyl solution.

However many patients treated by this drug have not been cleared of the cysts. Tuane and Taylor who have treated 3277 post dysenteric patients encountered 366 carriers. Sixty seven of these cleared up without treatment 215 were cleared of the cysts with emetine bismuth iodide and 84 failed to clear up.

Manson Bahr (1936) believes that the best results in the treatment may be obtained by combining this drug with y-tren (see below).

A more recent drug known as emetine periodide (E.P.I.) produced by Martindale is said to be less toxic than emetine bismuth iodide but the efficacy and effects of this drug have not yet been widely tested.

Many clinicians have felt that great caution should be exercised in the employment of emetine on account of its toxicity and in recent years many attempts have been made to secure as efficient a drug for amoebiasis which is less toxic than emetine and its compounds. Certain oxyquinoline derivatives and organic arsenical compounds have been especially studied and employed.



*Iodine oxyquinoline sulphonic acid* compounds have been widely used known as yatren (Bayer) sodium iodoxyquinoline sulphonate (Chiniofon U S P), quinoxyl (Burroughs Wellcome), anayodin (U S A), and dysentulin (Germany). These drugs contain about 26 per cent to 28 per cent of combined iodine, upon which depends their efficiency in treatment. Chiniofon (yatren) is supplied in pills or tablets each containing 0.25 gram (4 grains) and the dose for an adult is 3 to 4 pills, 3 times a day for a period of 8 to 10 days. The full dose sometimes causes severe diarrhoea so that it is recommended to commence with a smaller dose and increase it if it is well borne. The writer has had little experience in the use of these preparations except with yatren when it was first manufactured. However the drug has been widely used. Muhlen (1929) in Germany after 8 years of use regards it as the most satisfactory available remedy for the treatment of chronic amoebic dysentery and its sequelae, such as ulcerative colitis, membranous colitis and spastic obstipation. It is recommended that the drug be given both by mouth and in enemas. Manson Bahr believes that yatren acts best when the acute symptoms have been controlled by emetine, and in conjunction with that drug. He believes in order to obtain permanent results yatren must be given by the rectum in the form of a rectal injection of 227 cc., (8 ounces) of a 2.5 per cent solution of yatren, such an injection being given after a previous one, an hour before, of 1 pint of a 2 per cent sodium carbonate solution.

Craig (1937) believes that in the treatment of carriers chiniofon (yatren) is the drug of choice. He believes a single course of treatment is usually curative in carriers without symptoms but the course may be repeated if necessary after an interval of 2 weeks has elapsed. However rarely, more resistant infections have been encountered and he then recommends such drugs as vioform or carbarsone.

Vioform (iodo chlor hydroxy quinoline) contains between 37-41 per cent of iodine and about 12 per cent of chlorine. It is dispensed in gelatine capsules each containing 0.25 gram (4 grains) of the drug and one capsule is given 3 times a day for 10 days. After an interval of 1 week the same dose is repeated for another 10 days. This drug is said to be a more effective amoebicide than the other oxyquinoline derivatives. It is irritating to the rectal mucosa and cannot be used for retention enemas.

**Toxicity**—These drugs are excreted in the urine and can be recognized by the oxyquinoline test: a green color with perchloride of iron. While they have been said to be non-toxic abdominal colic headache diarrhoea, nausea palpitation and dyspnoea have been sometimes noted after administration of full doses. Animals killed by a large single dose show some degree of liver necrosis. Dyckerhoff has emphasized the damage done to the liver after yatren (Chiniofon) especially if it is given intravenously. The lesions bear a striking resemblance to those of subacute yellow atrophy as seen in arsenical and chloroform poisoning. He carried out experiments in rabbits using a 5 per cent solution of

yatren In this strength in normal animals very little change was noted If however by diet the glycogen content of the liver was reduced then the yatren produced the lesions mentioned above He emphasizes that in man before use a careful preliminary examination and determination of the bilirubin content of the blood should be made The drug evidently should not be used if there is any evidence of hepatic or renal damage

Among recent reports on the use of yatren Chopra Sen and Gupta (1937) have treated 50 cases 57.9 per cent of those showing vegetative forms of amoebae in the stool were pronounced cured and 62.9 per cent of the cases showing cystic forms of the parasites Hakansson (1938) states that the records of well controlled results in the treatment by yatren have indicated a high percentage of failures to eradicate the disease Manson Bahr (1936) believes that a combined treatment of yatren (by enema) and emetine bismuth iodide gives by far the best and most permanent results in treatment He has employed such treatment in over 300 cases with only 2 relapses

Another quinoline preparation has more recently been prepared diodoquin (5.7 diodo 8 hydroxyquinoline) In this preparation the sodium sulphionate radical of chiniofon has been replaced with the second iodine atom forming a double iodine compound It hence contains an increased amount of iodine 63.9 per cent Tenny Silverman and Hummel (1939) and Craig (1940) have advocated its use The compound is prepared in tablets each containing 0.21 gram (3.2 grains) and the dosage is from 7-10 tablets per day according to the severity of the disease Craig recommends that the drug should be tried as a prophylactic D Antoni (1943) has treated 84 cases of amoebiasis with Diodoquin of which 81 were cured and there were only 3 failures of the drug to effect permanent removal of *E. histolytica*

With reference to the use of iodine compounds Castellani (1935) has recommended the use of iodoform in the treatment of subacute and chronic cases of amoebiasis After a light or fluid diet and a purge of magnesium sulphate the iodoform is given in keratinized capsules each containing 0.05 gram ( $\frac{3}{4}$  grain) 1 or 2 capsules 3 to 4 times daily for 12 to 15 days After an interval of a week the course may be repeated Castellani states the drug may also be given in enemas 0.2 to 0.3 gram in 300 cc of water Scott (1937) and Radna (1938) have also reported favorably upon such treatment and with slightly larger doses No toxic symptoms were noted in some 28 cases

**Arsenic Compounds**—Various compounds of arsenic have also been widely used for treatment but following their use acute or chronic cases of poisoning are not rare sequelae Acetarsone (stovarsol) treparsol and carbarsone have been especially recommended Of these carbarsone is said to be less toxic and more actively amoebicidal Its use was particularly advocated by Reed Anderson David and Leake and Johnstone (1932) This drug is absorbed from the gastro-intestinal tract and excreted in the urine It is recommended to be given orally in dosage of 0.25 gram twice daily for 10 days and may be also used in 2 per cent solution for retention enemata In regard to its toxicity Anderson and Reed noted only one instance of carbarsone toxicity in 330 cases except for 7 cases having gastric distress Smithies (1934) however reports

4 cases of severe toxic reactions including dermatitis of the exfoliative type laryngeal and pulmonary oedema faulty vision due to papillitis and retinal oedema oedema of the ankles and wrists and enlargement of the liver and spleen also in several other patients nausea and vomiting and aggravation of diarrhoea and in one patient a slight icterus With one exception these patients had taken small amounts of carbarsone only from 2 to 20 capsules (0.5 to 5 grams) Brown (1935) reports 2 cases of toxic erythema and 1 case of neuritis Epstein (1936) has reported a death due to carbarsone poisoning which occurred after the administration of only 0.083 gram per kilogram of body weight during 10 days The patient was a woman 55 years of age At the autopsy there was acute fatty degeneration of the liver and exfoliative dermatitis The kidneys showed some tubular necrosis Guinea pigs rabbits and cats which succumbed to toxic doses of the drug all showed tubular necrosis of the kidneys Evidently the use of this drug is contraindicated in hepatic and renal disease

Hakansson (1938) has employed carbarsone in the treatment of 35 inmates in an asylum for the insane and 10 members of the laboratory personnel and their families In the former there were 6 cases of acute amoebic dysentery and 29 carriers In the latter all were carriers Only one patient showed untoward effects with jaundice and glycosuria after the ingestion of 0.5 gram daily for 20 days a total of 10 grams He eventually recovered Colic pains in the epigastrium were noted in one case and perhaps some of the insane suffered gastric and intestinal distress In the carriers even the small dose of 0.5 gram 2 capsules daily cleared the stools of *E. histolytica* in 2 to 3 days The drug was given for 4 weeks to 4 cases of acute amoebic dysentery after the stools had been cleared of *E. histolytica* by larger doses In 2 cases it failed to keep the stools negative and in 1 case it did not prevent a return of the dysentery while the drug was being taken However the large daily dose of 0.25 gram per kilogram of body weight used in 5 instances of acute amoebic dysentery was strikingly effective The blood and mucous soon disappeared from the stools The final results showed that in 20 of 35 treatments the stools were negative throughout the year while in 33 the stools became again positive during the year The author believes that in not a few instances reinfection had occurred He points out that even a large daily dose of 0.25 gram per kilogram of body weight i.e. 7 capsules daily for an adult weighing 70 kilograms given for 10 days may fail to cure some carriers This same dose however may promptly relieve the dysentery and bring about a clinical cure and eradication of the infection in some cases

**Other Methods of Treatment** — *Bismuth subnitrate* has been used in years past in the treatment of amoebic dysentery However it has a low amoebicidal power Its value in some cases has probably been largely due to the effect in controlling diarrhoea and bringing about chemical changes in the contents of the large intestine, sometimes detrimental to the life of the amoeba

Reports have also been made of the use of several alkaloids for the treatment of amoebiasis. Among them are conessine from kurchi bark, Kurchi bismouthous iodide (*anabin*) a glucoside obtained from *Castela nicholsoni*, *ritanol* a derivative of acridine and *Brucea sumatrana* from kosam seeds. None of these drugs has had an extended use and their value has not been conclusively demonstrated.

In very serious cases, particularly when gangrenous changes in the intestine may be present, the operation of appendicostomy has been recommended, following which a catheter is inserted and the large intestine irrigated with a 1 per cent solution of bicarbonate of soda to wash away the mucus and later with a boric acid solution. Castellani and Muller also recommend appendicostomy and irrigation in gangrenous cases. Phillips, however, is not enthusiastic in regard to this treatment and points out that its success has not been very great in many cases. Ross states that appendicostomy did not give encouraging results during the World War.

**General Treatment**—Patients with acute onset or acute exacerbations of the disease should be confined to bed. In the most severe forms when very frequent bloody mucous stools are being passed, the diet should at first consist of nothing but rice or albumin water. Later milk may be added. Rest is most essential and for this hypodermic injections of morphia sulphate gr  $\frac{1}{4}$  (gram 0.016) may be given every 3 or 4 hours. Its use should be pushed if necessary. Local treatment in this stage is contraindicated but treatment with emetin should be immediately commenced. Apart from this the essential point is to secure rest for the patient and for the acutely inflamed bowel. If this can be accomplished the condition usually improves. If the patient be seen before the symptoms are very acute a saline purge may be given but if the severe symptoms have set in this is contraindicated. McCay (1938) justly emphasizes again that the old purgative treatment in acute cases of dysentery by giving drachm doses of magnesium and sodium sulphate every 4 hours or so until the stools become completely watery is wrong; that an inflamed organ should not be stimulated and that the bowel needs to be given rest with opium and belladonna so that the lesions can be given time to heal. Where any intestinal irritation exists the diet should be restricted and not until the stools appear perfectly normal should general diet be permitted. In cases of moderate severity it is often advisable not to confine the patient entirely to bed for the reason that patients are likely to regain their strength better when up. Abdominal pain may be relieved by turpentine stupes and hot fomentations or if severe opium may be administered. When ulcers exist in the rectum and there is much tenesmus local treatment with argyrol or some other astringent or antiseptic substance may be applied through the speculum after the administration of a small enema containing cocain or morphin. Manson, Bahr and Gregg (1921) have employed the use of the sigmoidoscope both as an aid to diagnosis and to treatment in amoebic dysentery. Enemas of starch and opium sometimes have a very soothing effect. In connection with treatment radioscopy has sometimes been employed in determining the

localization of the larger ulcers bismuth sub nitrate being administered for several days before the photograph is taken with the hope that it will localize particularly in the lesions

Manson Bahr (1940) reports that X ray diagnosis has been tried on an extended scale at the Hospital for Tropical Diseases in London. Occasional filling defects are observed in the caecum but similar appearances are seen in other forms of dysentery and colitis. He adds 'It is disappointing to record that only unsatisfactory assistance can be obtained by this method for diagnosis of the infection'

If anemia is advanced, some iron preparation is necessary and where there is lassitude and anorexia a course of strychnin is often of value

Dunn has reported cases of dysentery which after prolonged courses of unsuccessful treatment by various drugs and other forms of treatment were put upon a full diet rich in vitamins and which almost immediately began to increase in weight and all symptoms of the disease vanished

### PROPHYLAXIS

In prophylaxis one should consider the sections on the Mode of infection and spread of the disease by the cysts of the parasite described on p 491 and the Resistance of amoebae to physical conditions and chemical substances ' p 490

The cysts do not survive when thoroughly dried. Hence infection occurs when they are in a moist stage and there is considerable evidence that amoebic infection is often waterborne. As ordinary chlorination of drinking water does not destroy the cysts, in the tropics drinking water should be boiled. However thorough sand filtration will eliminate the cysts from water. In view of the danger of wide spread outbreaks of infection due to defective plumbing such as was recently dramatically illustrated in connection with certain hotels in Chicago there should be more careful inspection provided for by public health officers and sanitary engineers of the plumbing of hotels, railroad stations and other public buildings. The plumbing hazards in connection with this epidemic of almost a thousand known cases were found to include back syphonage from sanitary fixtures to the water lines, leakage of sewer pipes into basements and even cross connections between sewer pipes and water pipes made by careless or ignorant plumbers.

Raw vegetables such as lettuce, radishes, strawberries etc. or other foods that may have been exposed to faecal contamination or to flies should be avoided. Such vegetables in countries where there is use of night soil for fertilization or which are grown on ground more or less public and subject to ordinary pollution are usually dangerous. Laws should be enacted against the use of human excrement for fertilization. Chandler found in parts of India that it was customary to soak uncooked vegetables in a potassium permanganate solution for an hour but it was very difficult to see that this was carried out efficiently by native cooks. All food should be protected from flies and cockroaches and these insects should be destroyed as far as possible.

There should be proper sanitary disposal of excreta and it is important that stools from cases of amoebic dysentery should not be left uncovered where flies may reach them but immediately treated with cresol

Transmission by the hands of carriers to food is probably not common and careful scrubbing of the hands with soap and water will probably eliminate the danger a carrier may be Nevertheless it is inadvisable that cyst carriers should be employed as cooks waiters etc and they should not be retained as servants until attempts have been made to free them of the cysts by treatment

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## Chapter XV

# LIVER ABSCESS AND OTHER COMPLICATIONS OF AMOEBIASIS

Abscess of the liver is one of the most frequent and serious complications of amoebiasis. The condition has been known since ancient times and Hippocrates reported its surgical treatment. Galen observed its association with dysentery. Koch 10 years after Loesch (1875) had reported amoeba as the cause of dysentery in man found the organism in the pus from a patient dying with tropical abscess of the liver. Kartulis also in 1887 noted the presence of amoebae in liver abscess pus and Osler in 1890 found amoebae in a liver abscess and in the faeces of a case of dysentery in the United States. Its incidence varies in different localities. Councilman and Laffleur who analyzed 1429 cases of amoebic dysentery found 21 per cent complicated by abscess of the liver. However Kartulis encountered 35 per cent of 500 fatal cases in Egypt with amoebic abscess of the liver. Strong and Musgrave in the Philippine Island in 100 autopsies largely upon Americans found abscess of the liver in 23 per cent while Clark in Panama in a series of 186 fatal cases found abscess of the liver in 51 per cent. Since the introduction of efficient treatment for amoebic dysentery it has become a rarer complication. Brown and Hodgson (1938) emphasize that it is rare in the north temperate zone. Tao (1931) in China in 1000 cases of amoebic dysentery found only 1.8 per cent with liver abscess and Craig (1934) in 745 cases found 5 per cent with liver abscess.

**Geographical Distribution**—The distribution of liver abscess of the type known as tropical abscess and more common in warm countries coincides with that of amoebic dysentery. It is particularly prevalent in those centers of amoebic infection in the tropics where there are many white men having little knowledge of the conditions necessary for the maintenance of health. In liver abscess as with blackwater fever it is education rather than acclimatization that brings about a diminution of the incidence.

For several years subsequent to the American occupation of the Philippines amoebic dysentery and liver abscess were common but in more recent years liver abscess has become rare in Americans and amoebic dysentery much reduced in prevalence. More temperate living may afford less deposition of fat in the liver and greater resistance to infection. Then too the more precise diagnosis and more scientific treatment of amoebiasis during the present century have contributed enormously to this lowered incidence of amoebic abscess of the liver.



## ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—The dislodgment of amoebae containing material from amoebic intestinal ulcerations and the plugging of the portal capillaries by such emboli may constitute the starting point of a liver abscess. The exciting cause is *Endamoeba histolytica* which in the liver produces a gelatinous necrosis similar to that in the submucosa of the large intestine or appendix. This pathogenic amoeba is fully described under amoebic dysentery in the previous chapter.

**Epidemiology**—Statistics in regard to obtaining a history of amoebic dysentery in liver abscess cases are as follows:

500 cases of abscess with history of dysentery in	60 per cent (Kartulis)
444	59 per cent (Zancanol)
500	85 per cent (Kelsch and Kiener)
63	90.5 per cent (Rogers)
38	85 per cent (Seamen's hospital autopsies)
50	60 per cent (Manson Bahr)

The liver abscess may develop at any time during the course of the intestinal infection. Not uncommonly it occurs after all symptoms of dysentery have ceased for a long period of time or indeed, sometimes



FIG. 131.—Large solitary amoebic abscess of the liver.

before any noticeable intestinal symptoms have developed. In some of the fatal cases of liver abscess there have been no evidences of intestinal lesions at autopsy and no history of dysentery during life. In the majority the abscess becomes evident in the first month after onset of dysentery.

Amoebic liver abscess is exceedingly rare among children under 10 years of age and probably 10 times less common among women than in men. However amoebic dysentery is not uncommon among women.

It is proportionately rare among natives. Thus in the native army in India the proportion of deaths from liver abscess to the total mortality in 1804 was only 0.6 per cent whereas in the British Army it was 7.4 per cent. Man for man the relative liability of the European to the native soldier was 95.2 to 4.8. Manson Bahr 1940 points out that this proportion holds in spite of the fact that a larger proportion of the native soldiers are infected with *E. histolytica*.

Of 40 cases of liver abscess Waring noted intemperance in 67.5 per cent and authorities generally insist upon the importance of the excessive use of alcohol as a predisposing factor. Other dietetic excesses and exposure seem also to predispose to the condition.

As to the proportion of cases of amoebic dysentery which give rise to liver abscess only the statistics of those who have differentiated between bacillary and amoebic dysentery are of any value. Such statistics would indicate that about 0 per cent of the cases of amoebic dysentery are complicated by liver abscess.

The incidence of liver abscess in the British troops in India for 30 years has been reported as follows:

	Liver abscess rate per 1000	Deaths per 1000
1897-1900	2.50	1.42
9.7-1909	1.80	0.76
1910-1919	0.715	0.6
1920-1924	0.52	0.164

### PATHOLOGY

The most common seat of the abscess is the upper and posterior portion of the right lobe. Ordinarily only one such abscess is found but in at least one third of the cases the abscesses are multiple, 2-3 or more large cavities being present. Rarely several hundred small abscesses may occur.

It seems to be clear that the amoebae usually invade the liver through the portal vein and are carried by way of the upward current into the liver localizing in the liver capillaries. The parasites are frequently found lying in the veins of the submucosa and in the portal capillaries and veins. It has been suggested that another method of transmission may occur and that direct infection of the anterior surface of the liver may sometimes take place the amoebae passing from a deep seated ulcer in the hepatic flexure through the peritoneum and anterior surface of the liver. In a few instances abscesses have been found on the anterior surface of the liver suggesting such an origin. It has also been suggested that the amoebae might possibly gain entrance by way of the bile duct. However bile is toxic to amoebae also it is not probable that amoebae would be present in the small intestines in the locality of the duct.

In many instances before suppurative lesions have occurred there may be a general congestion and enlargement of the liver. This may be confined to one lobe or even to part of a lobe. In cases which have succumbed from the accompanying dysentery, nodular areas measuring from one to several centimeters and grayish in color may be detected. They represent early areas of necrosis. In other instances the necrosis is more advanced and in the center reddish gummy or liquid pus is present. The larger abscesses are formed by massive necrosis of portions of the wall and partly by the formation of softening of additional foci in the neighborhood and subsequent breaking down of the intervening septum. The



FIG. 132.—*L. histolytica* in terms of portal vein. (Army Medical Museum 43321)

character of the pus changes becoming more liquid during the evolution of the abscess.

In about 50 per cent of the abscesses bacteria may be obtained by cultivation when sufficient material is inoculated. The remainder are apparently sterile in regard to microorganisms except for the presence of amoebae. *Staphylococci*, *streptococci*, and *colon bacilli* are not infrequently encountered. It is obvious why bacterial infection so frequently occurs, as these organisms have probably the same opportunity for entering the liver as the amoebae. Undoubtedly the pus cocci when present exert an injurious influence upon the hepatic tissue but there can be little doubt that the amoebae play a most important part in the formation of the abscess. This is demonstrated by the very different character of the amoebic and pure bacterial variety of abscess. The smaller amoebic abscesses consist of thick, glairy yellowish masses of mucus which are not

fluid In the larger abscesses the contents are more liquid and of a creamy gelatinous purulent consistence In color they are yellowish grayish red brownish red or greenish from the adjacent mixture of bile Frequently shreds of necrotic liver tissue are mixed with the fluid portions Microscopically one is struck usually with the absence or presence in small numbers only of polymorphonuclear leucocytes The contents consist of granular material containing fragments of cells swollen degenerated liver cells red blood corpuscles fat globules cholesterol crystals and amoebae The latter are sometimes difficult to find in the pus but can almost invariably be obtained in scrapings made from the abscess wall In the abscesses in which no bacteria are found microscopical examination shows that the amoebae have apparently given rise to the necrosis and liquefaction of tissue without any very pronounced inflammatory reaction The contents of such abscesses consist chiefly of the debris of liver tissue with relatively slight admixture of leucocytes In the early liver abscesses many liver cells can still be seen There is oedema of the surrounding tissue The amoebae are found in the edge of the living tissue and there are a few mononuclear phagocytic cells in the vicinity Later the necrotic lining of the cavity loses its recognizable constituents and shows only a mass of nuclear fragments with a few leucocytes Councilman and Laflour have also described a widespread necrosis of the liver cells situated around the central veins of the lobules and scattered throughout the liver They suggested that this was due to soluble chemical products of the amoebae The striking feature is the absence of leucocytic infiltration which usually accompanies suppuration of bacterial origin The amoebae are often found only in the margins of the living and not in the necrotic tissue unless the abscess has been opened to the air MacCallum points out that this is because of their need of oxygen and he emphasizes that when an amoebic abscess is first opened frequently no amoebae are found in the pus until the following day After the cavity has been exposed to the air however the pus may be full of active amoebae The abscesses may reach a large size before rupture or evacuation occurs and may contain several liters of pus Large areas of liver tissue are thus entirely destroyed leading sometimes to functional disturbances

#### SYMPTOMATOLOGY

Clinical Course —The liver abscess may develop so insidiously that it is frequently overlooked and perforation may be the first indication It should be borne in mind that with amoebic liver abscess there is not a single symptom that is constant and proof that the liver is involved may be very doubtful The general condition and appearance of the patient with the progress of the case rather than any single clinical symptom often suggest the diagnosis which may sometimes be confirmed by aspiration A history of previous dysentery may suggest the condition but an absence of such a history cannot exclude the diagnosis of liver abscess If the onset is acute the diagnosis may be simplified Rogers recognizes a condition which he terms the pre suppurative stage of amoebic

In many instances before suppurative lesions have occurred there may be a general congestion and enlargement of the liver. This may be confined to one lobe or even to part of a lobe. In cases which have succumbed from the accompanying dysentery, nodular areas measuring from one to several centimeters and grayish in color may be detected. They represent early areas of necrosis. In other instances the necrosis is more advanced and in the center reddish gummy or liquid pus is present. The larger abscesses are formed by massive necrosis of portions of the wall and partly by the formation of softening of additional foci in the neighborhood and subsequent breaking down of the intervening septum. The

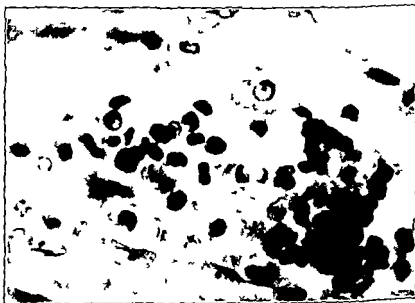


FIG. 132.—A histological section of liver tissue in terminal of pyralis (Army Medical Museum No. 43321).

character of the pus changes, becoming more liquid during the evolution of the abscess.

In about 50 per cent of the abscesses bacteria may be obtained by cultivation when sufficient material is inoculated. The remainder are apparently sterile in regard to microorganisms except for the presence of amoebae. Staphylococci, streptococci and colon bacilli are not infrequently encountered. It is obvious why bacterial infection so frequently occurs, as these organisms have probably the same opportunity for entering the liver as the amoebae. Undoubtedly the pus cocci when present exert an injurious influence upon the hepatic tissue but there can be little doubt that the amoebae play a most important part in the formation of the abscess. This is demonstrated by the very different character of the amoebic and pure bacterial variety of abscess. The smaller amoebic abscesses consist of thick, glairy yellowish masses of mucus which are not

or it may progress so as to cause bulging on the right side. Occasionally a swelling may be observed over the sixth or seventh rib. The movements of the right side of the chest during respiration may be limited and the right rectus may show rigidity. Radioscopy may also reveal fixed diaphragm on the right side. Percussion and auscultation frequently give no information of the condition though if the abscess is large percussion may reveal an increase in hepatic dullness. Friction may be heard over the liver when the peritoneum is involved. Crepitation at the right base of the lung with dry cough and shallow respirations may suggest the condition.

The blood examination usually shows a moderate leucocytosis of from 12 000 to 20 000 or even more cells per cubic millimeter.



FIG. 134.—Liver abscess. X-ray photograph taken from the dead shadow produced by the enlargement of the liver. (Rug and Urverth ft. Bé lére)

The relative leucocyte count may show an increase in the polymorphonuclear leucocytes. Manson Bahr (1936) in his series of cases found the mean average of the differential count to be 70.8 per cent polymorphonuclears, 22.2 per cent lymphocytes, 6 per cent large mononuclears and 1 per cent eosinophiles.

Functional disturbances have been suggested as sometimes being of assistance in determining the presence of liver abscess. However the results recently obtained with the bromsulphalein and other liver function tests as an indication of hepatic disease and liver abscess have frequently proved unsatisfactory. Brown found the bromsulphalein test indicated liver damage in 8 of 13 of his cases of abscess.

hepatitis in which amoebae from dysentery lesions have lodged in the portal capillaries of the liver, but in which actual abscess formation has not taken place. There may be a low remittent fever and a leucocytosis in which polymorphonuclear leucocytes are but little increased in percentage. At this stage Rogers believes that the disease may often be cured by emetin and liver abscess avoided. The differential diagnosis between the presuppurative and the suppurative stages is however often very difficult to obtain without puncturing. If chills and sweating are present and the condition does not improve by emetin suppuration may be suspected.

Fever, pain, enlargement, and functional disturbances are the more frequent indications of liver abscess and may point to the advisability of exploratory puncturing and discovery of the pus. Fever is usually present at some time but is often insufficient to attract attention. Some times it is irregular, from  $100^{\circ}$  to  $102^{\circ}\text{F}$  and it may be septic in type

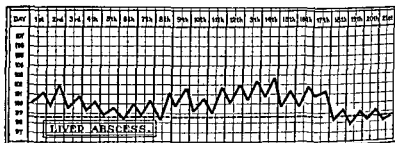


FIG. 133.—Temperature chart of liver abscess

rising in the evening to  $103^{\circ}$  or  $104^{\circ}$ . Chills and sweats may occur, and the symptoms more or less closely simulate those of malaria. The conjunctivae are sometimes slightly tinged with yellow. Distinct jaundice is rare. Persistent vomiting may occur. The skin frequently assumes a sallow color and becomes pale and yellow. The facies may suggest the diagnosis. In certain cases emaciation occurs rapidly; in others the flesh is well retained. The appetite usually disappears and the tongue becomes coated. Albumin may appear in the urine together with an excess of urobilin and of nitrogen eliminated as ammonia. Pain is a variable symptom. Brown (1938) at the Mayo Clinic, has found pain in the right lower thorax the most frequent symptom. It may occur in the right shoulder when due to irritation of the branches of the phrenic nerve or over the hypochondrium or epigastrium. Brown found it in the right shoulder in 8 of his cases and in the left in one. Van Gorder and Chen (1931) in Chinese cases noted pain in the right upper quadrant as the most common complaint in 39 of 48 cases. When not present spontaneously pain may be elicited on pressure over the liver. Enlargement may be detected by physical examination or by radioscopy. The enlargement is usually upward and may reach as high as the angle of the scapula.

prognosis is fairly favorable. Hodson recently has reported two cases of liver abscess which had ruptured through the lung in which the patients recovered at once after treatment with emetin. Rupture into the peritoneal cavity and pericardium is practically always fatal. Sambuc found that in his cases where only one abscess was present the mortality was 23 per cent with 2 abscesses 45 per cent with 3 abscesses, 90 per cent and with more than 3 100 per cent.



FIG 135—Amoebic abscess of the liver. Photograph of a patient with a large amoebic abscess of the right lobe of the liver. (After Channing and Vignani, Dept. of Surgery, P. U. Medical College, P. P. G.)

### PROPHYLAXIS

**Tropical Hepatitis and 'Tropical Liver'**—Although the statistics would indicate that a history of amoebic dysentery had been obtained in only from 60-90 per cent of cases of liver abscess yet when we consider that amoebic lesions of the large intestines have been frequently noted at autopsy in those who had never shown symptoms of dysentery during life the evidence inclines one to believe that amoebic lesions of the large intestines or appendix were at some time a factor in the production of the liver abscess. Consequently a history of amoebic dysentery is one of the most important points to consider in the making of a diagnosis of tropical liver abscess.

There is also much evidence to be obtained from statistics and other wise to support the view that amoebic and other infection of the liver is much more likely to occur in a person whose liver has already been functionally impaired. To this condition the designation tropical congestion of the liver or simply tropical liver has frequently been applied. There is much to support the view that in the tropics the intestines and liver probably more often take the brunt of those conges-



## DIAGNOSIS

An absolute diagnosis can often only be made by aspiration and finding the amoebae in the pus. A needle having a sufficiently large caliber to transmit the thick pus should be used the punctures being made through the skin over the suspected point or points.

*Pus from liver abscesses* is characterized by its very viscid tenacious consistence and chocolate brown color. It is usually streaked or mixed with fresh blood in varying degrees. Rarely it is creamy and yellow. This pus gives to the sputum its anchovy sauce appearance in cases in which the abscess ruptures into a bronchus. Microscopically such pus consists largely of granular cellular detritus red blood cells and occasionally large phagocytic cells and a few leucocytes. Pus cells however are usually sparse or absent unless there is secondary bacterial infection. It may contain cholesterol haematoidin or Charcot Leyden crystals. Motile amoebae may be numerous but are usually sparse or absent in material obtained by aspiration or in the discharge for a few days after drainage has been established. Cysts are never found.

## PROGNOSIS

The outlook before the discovery of more appropriate treatment was usually grave and the mortality in the tropics was generally over 50 per cent. Under surgical treatment usually not more than one third recovered. However owing to the use of emetine in the treatment of amoebic dysentery and liver abscess particularly in its pre suppurative stages and the improved surgical methods for evacuation of pus and drainage the mortality has been greatly reduced. Thus Cort among 530 cases of amoebiasis in Siam reports 97 cases with amoebiasis of the liver with but one death. All were treated with emetine the large abscesses being also aspirated. Van Gorder (et al) observed a mortality of 20.8 per cent (in 48 cases). The mortality in the 25 operated cases was 32 per cent and in the 22 non operated cases 99 per cent. Brown, who operated upon 18 cases at Rochester had but two deaths. All of the others recovered after operation and anti amoebicidal treatment. There are some physicians who regard emetine as showing an almost complete specificity for the amoeba lodging in the liver capillaries and claim little mortality from the use of emetine alone but it stands to reason that the evacuation of an abscess as a stage of the treatment must favor the convalescence and restoration of the liver to its satisfactory functioning. It is with the solitary abscess of the liver that we get our lowest death rate—with multiple abscesses this increases. Spontaneous rupture of the abscess frequently occurs if the patient lives long enough and is not operated upon. This is most common into the lower lobe of the right lung. Rupture into the abdominal cavity causing general peritonitis is also frequent. Cyr's statistics of 159 cases show that rupture occurred into the lungs in 59 into the pleural cavity in 31 into the peritoneal cavity in 39 into the intestine in 8 into the stomach in 8 into the vena cava in 3 into the kidneys in 2 into the bile ducts in 4 into the pericardium in 1, externally in 2. The duration of liver abscess is very variable being from a few weeks to many months. In those cases in which the abscess ruptures into the lung the

The prophylaxis of amoebic disease of the liver is the same as that for amoebic dysentery plus avoidance of anything which reduces the functional power of the liver such as over feeding alcoholic excesses etc. It is well to remember that abscesses may occur months or even 2 or 3 years after an attack of amoebic dysentery consequently it has been suggested that if there are symptoms suggesting hepatic involvement treatment with emetine should be considered. There is nothing so important in the prevention of liver abscess as accurate and early diagnosis of amoebic colitis and its intensive treatment by every means at our disposal. Liver involvement may follow various diseases and malaria may be coexistent so that in addition to avoidance of excesses and chilling of the body any other disease processes present should be treated promptly. Judicious exercise before sunrise is usually more important than the taking of purgatives. For treatment see p 539.

#### OTHER COMPLICATIONS

**Abscess of the Lung**—Next to the intestine and liver the lung is the organ most frequently invaded. The abscess may be secondary to one



FIG 136—Hep to-pulmon ry amoebic absc

in the liver opening into the lung or a primary abscess of the lung may occur independent of hepatic abscess. The amoebae may also invade the lung from the hepatic veins and the abscess may result in this manner rather than by direct extension of the process from the liver. Bunting was able to trace emboli containing amoebae from a thrombus in the hepatic vein which also contained them and to show that this embolism of the pulmonary arteries had produced an amoebic abscess of the lung. Amoebic ulcers of the intestines had at the time already healed. The lung abscess is similar in character to that of the liver. Rupture of liver

tions which in temperate regions are borne more by the thoracic organs. In temperate climates, excesses and exposure to debilitating influences may result in coryza or pneumonia. In the tropics, they frequently result in diarrhoea and congestion of the liver.

Tropical liver may be recognized by vague digestive troubles, high colored urine, loss of energy, irritability, with a sensation of fullness in the region of the liver which is generally described by the patient's statement that he feels his liver. There may be pain referred to the right shoulder and the liver may be tender on palpation.

Manson in earlier years in the Far East probably came frequently into contact with this condition. Apparently repeated hyperaemic attacks and engorgement of the liver might constitute the first step towards a hypertrophic cirrhosis which, however, was frequently never materialized.

Manson summed up, in earlier years his studies with the following passage:

The young European who finds himself in the tropics for the first time is surrounded very often by luxuries in the way of food, wine, carriages and servants—luxuries to which he has not been accustomed perhaps in his home. At first the change, the excitement of novelty and the high temperature act as stimulants to appetite and the excessive loss of fluid by cutaneous transpiration creates a powerful thirst. Little wonder therefore that in such circumstances the youth, having the appetite and the opportunity of gratifying it, is apt to indulge in food and drink beyond safe physiological limits. He is made lazy by the heat; he cannot exercise during the day and when evening comes prefers lounging on the verandah or hanging about the club bar to walking or riding or games. Very likely he sits up late at night drinking and smoking so that in the morning he is too sleepy to ride out or to take any other form of exercise. And so it comes about that with a surcharge of aliment and alcohol and the diminishing activity of lung metabolism and excretion incident to the high temperature and muscular inactivity, a very large and unusual amount of physical work is thrown on the liver. With the large amount of work there is a corresponding hyperaemia. This may be considered the first stage of tropical liver—hyperaemia from functional inactivity. Up to this point it is a purely physiological condition. Pushed a step further this physiological hyperaemia passes into congestion with blood stasis and a consequent diminution of functional activity. Hyperaemia of a physiological type will be evidenced by an increase of functional activity and there will be a copious flow of bile, sometimes causing diarrhoea of a bilious character, particularly morning diarrhoea, but when the limits of physiological hyperaemia are passed and congestion of a pathological character sets in, the consequent arrest of function will be evidenced by pale stools, perhaps diarrhoea of a pale, watery, frothy fermenting character. Other symptoms of this condition are headache, furred tongue, scanty, high-coloured, loaded urine, a feeling of weight or fullness or even of pain in the region of the liver, and a probable extension of the area and other physical signs of enlargement of that organ.

Tropical liver among the upper classes of the Far East, as gout in the United States and parts of England, is a condition which the younger practitioners of today probably will much more rarely be brought into contact with.

By the discontinuance of alcohol and of the daily consumption of large amounts of rich and highly spiced foods, with treatment by phosphate of soda or sodium sulphate, together with general care of the health and exercise, the patient may often recover completely and the predisposition to abscess avoided.

earlier stages in which the pia matter may be affected distended and thrombosed vessels are seen with some oedema and amoebae in fairly large numbers. There may be leucocytic invasions of the tissues beneath and initial softening with haemorrhages. As softening takes place a cavity is gradually formed the contents consisting of broken down and degenerate nerve tissue which sometimes occurs in masses connected with the thrombosed vessels. The fluid is usually sterile bacteriologically though both aerobic and anaerobic organisms have occasionally been isolated. The symptoms of amoebic abscess of the brain do not differ as a rule from those of cerebral abscess produced by bacteria and they will therefore not be considered here in detail. Headache is very severe and lumbar puncture may not relieve it. Meningitis occurs only exceptionally and hence lumbar puncture generally yields a clear fluid. The toxic evidences of the suppuration are not prominent and there is usually little or no evidence of intracranial tension. The disease advances rapidly and death usually occurs from the sixth to the eighth day after onset. The patient loses consciousness and coma rapidly develops. In the cases reported by Runyan and Herrick death occurred within 48 hours. When the abscess bursts into the ventricle the course is very acute. Convulsions may occur and the temperature may rise. In Zancarol's case the first sign was a sudden attack of mania. In others coma may suddenly develop without preceding symptoms. Cases have been recorded in which the onset has been sudden with signs of Jacksonian epilepsy. The diagnosis of the nature of the abscess is frequently difficult. The history of previous dysentery or the presence of amoebae or cysts in the faeces may suggest the diagnosis. Lumbar puncture and ophthalmoscopic and blood examinations may assist in excluding cerebral lesions due to syphilis middle ear disease other septic conditions or meningitis.

**Peritonitis**—Local peritonitis may result from extension of inflammation from the ulcerations in the intestinal wall until the peritoneal coat is invaded with deposition of lymph fibrin and other inflammatory products on the surface. Patches of fibrous adhesions are frequent in chronic cases and it is the rule to find old localized areas or chronic adhesive peritonitis. These may cause abdominal soreness and pain. Peritonitis which generally proves fatal may follow perforation of a liver abscess or of an intestinal ulcer. Perforation of the intestine results generally from the base of a deep sloughing ulcer. The perforation frequently occurs in the caecum and the condition has sometimes been mistaken for one in which the appendix is involved. Perforation of the large bowel with acute peritonitis was found in 19 of 100 severe cases of the disease occurring particularly in soldiers on field service. Usually it is much rarer occurring only in 3 or 4 per cent of hospital cases. It is almost invariably fatal. Perforation sometimes occurs after adhesions have formed when a pericaecal or pericolic abscess may result. On the other hand perforation may take place retroperitoneally into the psoas muscle and may even open externally through the skin.

*Amoebic appendicitis* has frequently been reported the involvement of the appendix usually following an extension of the disease process

abscess is more common into the lung than elsewhere. It occurs in from 10 per cent (Kartulis) to 20 per cent (Rogers) of liver abscess cases. The diaphragm is usually adherent to the liver and to the base of the lung. The lower right lobe is affected. The diaphragm may or may not be visibly perforated. The formation of the abscess is usually preceded by irregular fever and an irritable cough. The respirations are frequently increased in number and painful and shallow. Before perforation of the abscess occurs the signs of pleurisy are usually present. The cough becomes more severe and expectoration appears. As the abscess advances definite dulness may be found with bronchial breathing or absence of respiratory sounds over the affected area. Radiographic examination may be of considerable diagnostic value by revealing a dense shadow in the affected part of the lung in addition to the fixation of the diaphragm so common in liver abscess. If the abscess discharges into the pleural cavity or into a bronchus the existing dyspnoea becomes less marked. Sooner or later the characteristic anchovy sauce like sputum appears in which can sometimes be found amoebae together with altered liver cells as well as alveolar epithelium elastic tissue fibers Charcot Leyden crystals and other elements. When rupture into a bronchus takes place the abscess may continue to discharge for months or the case may end fatally in a shorter time. In favorable cases the pus diminishes and the cavity cicatrizes but complete recovery may be delayed for over a year.

**Abscess of the Brain**—Hitherto abscess of the brain has proved an invariably fatal complication. It has been particularly studied by Kartulis, Jacob, Legrand, Phillips and Armitage. Legrand collected 45 cases from the literature in 1912 and he, Phillips and Armitage have recently reported 5 more cases. Europeans are most frequently affected. In 27 of the cases the disease originated in Egypt. Five however, occurred in patients who contracted dysentery in France, Germany or England without ever having left these countries. With four exceptions the patients were between 20 and 40 years of age. One case occurred in a girl of 5 and one in a boy of 14. Only 3 were in females. The preceding dysentery or liver abscess may have become cured perhaps weeks or even months before the abscess in the brain developed or the intestinal infection of the bowel may have remained latent. In the majority of the cases the liver or lung had been previously affected but in 2 at autopsy there was no apparent lesion in either. The abscess is usually single but may be multiple. In all but one of the cases it was in the cerebrum occurring most frequently in one of the hemispheres. It may rupture into the ventricle and cause acute symptoms. In one case it was situated in the cerebellum. Runyan and Herrick have reported 2 cases in Panama both of which followed operation upon liver abscess. They believe that traumatism of the liver abscess wall resulted in the extension of the infection to the brain.

The contents of the brain abscesses frequently resemble those of liver abscess, being reddish in color. Necrosed portions of brain tissue attached to the wall by thrombosed vessels may be found. The microscopic appearances also recall the condition seen in abscess of the liver. In the

**Rarer Complications**—Of the still rarer complications due to intestinal amoebae of which cases have been reported may be mentioned abscess of the spleen cystitis ovarian abscess salpingitis parotitis fistula disease of the buttocks and purulent or gangrenous subcutaneous infection of the skin in the vicinity of wounds subsequent to operations for liver abscess or upon the rectum or intestine (see p 500) Severe intestinal haemorrhage in which large amounts of pure blood are passed from the rectum may also occur Fatal intestinal haemorrhage may take place independently of liver abscess but when intestinal haemorrhage occurs in patients with liver abscess it is likely to be very severe and recurrent in character.

#### TREATMENT OF THE COMPLICATIONS

The treatment of amoebic hepatitis or of amoebic abscess of the liver if diagnosis is made early should consist of subcutaneous injections of emetine hydrochloride 0.065 grams (1 grain) a day for a period not over 12 days Reference has already been made to the treatment of the pre suppurative stage of amoebic hepatitis with emetine which according to Rogers and others is frequently successful However if emetine fails to arrest the symptoms and as soon as signs of suppuration are apparent the abscess should be opened and freely drained unless it has already perforated into the lung and is being freely discharged through a bronchus If the abscess has opened it may be irrigated frequently with quinin solution 1:1000 or 2 oz of an emetin solution 1:1000 may be injected into the cavity hypodermic injections of emetin also being employed at the same time Prior to operation an aspirating needle of sufficient caliber to transmit the thick pus is usually employed for location of the abscess The surgeon must be prepared to operate at the time these exploratory punctures are made and it should be borne in mind that such punctures are not without danger for fatal haemorrhage sometimes follows them Rogers points to the danger of post suppurative infection in India and recommends aspiration of the pus and the injection of quinin solution or of emetin by means of a special trocar with a flexible silver sheath Charles however prefers the open operation preceded by aspiration on the ground that drainage by these methods is not thorough Cope (1920) also advises the open method of free incision

The success that may be obtained by operation upon liver abscess is indicated by Brown and Hodgson 1938 Thus among the 18 patients operated upon at the Mayo Clinic there were only 2 deaths Fourteen of the patients operated upon were apparently well from 2 to 12 years later In one case there was evidence of a recurrence 6 years after operation In 14 cases in which there were clinical signs of hepatic involvement the cases were treated medically and the results of such treatment are unknown in 2 The remaining 12 remained apparently well 2 to 6 years later Treatment consisted of emetine injections followed by treparsol orally

Perforation of the bowel demands surgical aid if the condition of the patient warrants it Surgeons with wide modern experience such as

from the caecum. A definite diagnosis is very difficult during life, since the caecum, in addition, is usually extensively ulcerated.

Clark (1935), Craig (1936), Banerji, Chopra and Ray (1936) have emphasized the frequency of this complication. Clark found that in about one half of his autopsies where the caecum revealed amoebic ulceration the appendix was also involved in some manner but he does not list one case in which the appendix alone showed lesions and where some portion of the large intestine was not also involved. In an analysis of 60 cases of amoebic dysentery made by Craig 16 were found to have appendicitis. However, Craig points out that while it is evident that appendicitis may occur during the course of amoebic dysentery it should not be forgotten that many of the cases so diagnosed clinically do not



FIG. 137.—Amoebic appendicitis

have any inflammation of the appendix but suffer from symptoms simulating those of appendicitis.

Many cases of intestinal amoebiasis with ulceration of the colon and caecum have been operated upon for appendicitis with fatal results. During the recent Chicago epidemic of amoebic dysentery many of the fatal cases had been operated upon owing to the mistaken diagnosis of appendicitis during life. In fact, in 41 cases the diagnosis of appendicitis was made and the correct diagnosis of amoebic infection not recognized. Thirty-two of these cases were operated upon for appendicitis and appendectomy performed resulting in the death of 13 or 41 per cent. The results of operation on severe cases of amoebic infection are very frequently disastrous.

Sapero (1939) studied 216 cases of non dysenteric amoebiasis of which 100 were found to have different symptoms. A symptom complex simulating subacute or chronic appendicitis was the most commonly observed syndrome in the series. The complaints made by the individuals were frequently of a trivial nature. They were primarily referable to the gastro intestinal tract but none of them had blood or mucus in the stools.

In a series of 198 fatal cases at the Gorgas Hospital, Panama reported by Clark the appendix showed lesions in 76 or 38 per cent. Faust in 7 cases of amoebic infection found pinpoint lesions only in the appendix in 2

## SECTION II

### DISEASES DUE TO BACTERIA

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#### Chapter XVI

#### BACILLARY DYSENTERY

**Definition**—Bacillary dysentery may be defined as an acute infectious disease often characterized by sudden onset and by frequent mucous bloody stools accompanied by abdominal pain and intense tenesmus. It terminates either abruptly in death (usually in from 4–15 days) or by gradual improvement from which an early recovery takes place or the disease may apparently pass into a subacute or chronic stage. *Bacillus dysenteriae* the cause of the disease is present in the acute stages both in the discharges and in the lesions of the intestinal mucosa. It may be characterized anatomically by an acute diffuse inflammation of the large intestine leading to a superficial necrosis of the mucosa and usually by hyperplasia and haemorrhagic infiltration of the lymph follicles by induration and thickening of the intestinal wall by haemorrhagic swelling of the adjacent mesocolic glands and often by parenchymatous changes in the viscera. This description applies to the form of the disease as it usually occurs in tropical countries. In temperate climates in which the milder forms of infection are encountered the most important clinical symptom may be intestinal disturbances accompanied by diarrhoea.

#### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History**—Epidemics of dysentery have been noted since ancient times the widespread and fulminating nature of such outbreaks in times of war and famine having impressed observers in all ages. The disease is apparently referred to in the Ebers Papyrus (1600 B.C.). Herodotus mentioned an epidemic of dysenteric nature in the Persian Army and Hippocrates described the dysenteric syndrome. It has been well known in India since remote times.

While the etiology of amoebic dysentery was thoroughly investigated and its connection with amoebae fairly well established during the decade from 1880 to 1890 it was not until 1898 that Shiga isolated the causative organism of bacillary dysentery although a number of other bacteria had been previously erroneously described as the cause of the disease.

Bacillary dysentery tends to appear in extensive epidemics spreading over temperate as well as tropical and subtropical parts of the world. In this respect it differs from the amoebic form.

It is liable to follow the movements of armies in any part of the world and like typhoid fever its distribution is one of hygienic rather than geographical influence.



Cope agree that immediate abdominal section is necessary in the cases in which the general condition is good and the symptoms of perforation acute and that, on the other hand when the general condition is bad or indifferent non intervention is frequently justified by recognition of the fact that perforations are sometimes cured naturally that escape of the contents of the gut is often greatly limited by adhesions and that the bowel wall is frequently friable and unsuitable for suture. Local peritonitis without perforation requires rest and the application to the abdomen of ice or hot fomentations with opiates by the mouth. For serious haemorrhage complete rest is demanded morphia should be given and ice applied locally to the abdomen. Stimulants and subcutaneous or intravenous injections of saline solution should be employed only when their use is indicated. Adrenalin in doses of 10 to 15 cc of a 1:1000 solution has also been recommended. The injection of a solution of calcium chlorid or of horse serum has also sometimes given favorable results. For the treatment of brain abscess in addition to emetin treatment, extensive trephining is recommended, and the abscess should be sought for with a channeled sound and not with an aspiration needle since the pus is very viscid and flows with difficulty. When the abscess is localized it should be opened by operation. Morphia and bromids are indicated for the relief of headache and other cerebral symptoms. Abscess of the lung requires the usual treatment with emetine. X ray examinations may give information as to the advisability of surgical intervention for better drainage in case a liver abscess has ruptured into the lung. If the abscess has opened into the pleura rib resection may be performed and drainage thus secured.

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virulent form. Large epidemics are also more common in such countries. The occurrence of the disease in epidemic form is influenced by the sanitation of the region being more prevalent in communities where fly suppression and garbage disposal are not properly controlled. Even in more temperate climates the disease is apt to occur during the warmer months of the year. Sellards (1921) has pointed out that bacillary dysentery has never given rise to any great pandemics. The curves of seasonal distribution show the close relationship between temperature and the appearance of both bacillary and amoebic dysentery. (The maximum from July to September.) On the other hand there is often no seasonal maximum in countries where the cool climate or the sanitary conditions keep the number of cases very low (England, the Netherlands and Australia for example) and in those near the Equator which have no definite seasons (Guiana, Sumatra).

The disease was encountered in severe epidemic form in the Philippine Islands shortly after American occupation (1899-1900) and numerous outbreaks have been reported from Japan. Indeed the disease in epidemic form has been more commonly reported from Japan than from any other country where according to Shiga and others the mortality is often high (from 2 to 50 per cent). Yokoyama (1931) reports that in Darjeeling, Manchuria for the past five years it was the most prevalent disease—3.4 per mille with a mortality of 38 per cent.

Outbreaks also have been common in many parts of the tropics, notably in India, Indo-China, China, Ceylon, Malaya, the Philippines, Java, northern Brazil, Haiti, Panama, Puerto Rico, Egypt, Syria, Arabia, Palestine and in parts of the French and Belgian Congo. In India, Malaya and the Pacific Islands the disease is responsible for wide spread epidemics with high mortality. Manson-Bahr records in the Fiji Islands in 1884 an epidemic with a mortality rate of over 130 per thousand and in recent years the mortality rate there has varied from 58 to 128 per thousand.

**Dysentery and War.**—In times of war with large forces of soldiers bacillary dysentery tends to become the most important disease encountered by military surgeons. During the Civil War it may be recalled there were 85,000 cases of dysentery in the Federal Army. In its epidemic form as intimated it has been particularly a disease of armies in the field. It twice succeeded in having decimated the army of Napoleon in the retreat from Moscow. In the Crimean War the American Civil War the Franco-Prussian War the South African War the Philippine Campaigns the Russo-Japanese War a deeply northern World War it was very prevalent. In the last war while it occurred on all fronts it was the most important disease in the British armies in the eastern Mediterranean area, Macedonia and Gallipoli. In Macedonia Leedingham reported that bacillary dysentery of 5 to 83 per 1,000 and in Gallipoli it was said to be largely responsible for some 100,000 casualties. Abraham (1939) writes that bacillary dysentery and not the Typhoid fever the British troops of Gallipoli according to Primrose the average strength of the German army was 3,250,000 and 82,500 died of disease. Out of every 100 who died 4 deaths were from dysentery.

Colonel George R. Clender has for many years made study of dysentery and diarrhea in the United States Army and points out that (1941) the rate of dysentery and diarrhea gradually declined to 7 in 89,000 by 1917, rose to 400 in 898 Spanish War and 48 in 1915. Philippine Campaign declined to about 4 in 1915. There was a sharp rise to 87 in 1916 as a result of epidemic conditions in the Mexican Border mobilization. Roth suggests the rates dropped in the U.S. Army during World War I. They continued to 200 under unusual mobilization and extensions of training of 1914 when the rate was slightly over 37 per thousand per annum nearly double that of the previous period 1918-1930 inclusive. Callender (1943) has emphasized again the importance of dysenteries and diarrheas in military service in an article which should be read by all concerned with the subject. He shows that in the present war bacillary dysentery has risen in troops to heights equal to those of 1914-1918. The disease in the present war has been the most prevalent one in the British troops in the Middle East (Bulme 1944) as well as in the Italian and German troops (Gear 1944).

While it is more frequent and often more virulent in the tropics infections with various strains of dysentery bacilli are important factors in morbidity among infants and young children in whatever part of the

## Europe 1932

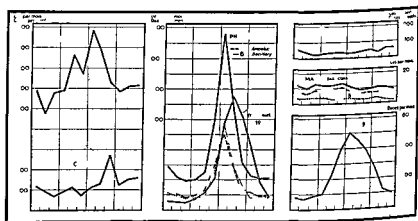
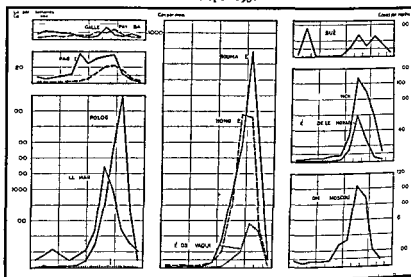


FIG 138—Seasonal distribution of dysentery cases reported (Epidemiological Intelligence Service of the League of Nations)

world the question has been investigated. The infection is also prone to prevail in lunatic asylums whether in temperate or tropical countries.

**Distribution**—Bacillary dysentery is characterized by a much greater tendency toward epidemic distribution than any other form of dysentery. The disease is not limited to any particular part of the world. In tropical and subtropical countries it occurs more frequently and often in more

these insects are comparatively rare. This occurs in the Near East and in Africa during the hot season when the majority of the fly larvae are destroyed by the rays of the sun. In the southern United States Kuhns (1913) has reported a camp outbreak of 1250 cases where infection was due to flies. The flies from the latrines, kitchens, etc. were examined culturally and one lot yielded *Shigella paradysenteriae* Boyd 88; the same organism was isolated from a number of the patients. The drinking water of the camp was chlorinated and the milk used was a canned product.

**Age and Sex**—Individuals of all ages are subject to dysentery, but it is most common in men between 20 and 30. It is not infrequent in young children. Outbreaks occur especially in those under 5 years of age.

**Bacteriology**—Many different bacteria had been erroneously described as the cause of dysentery in the years of 1882-1898. However, it was not until 1898 that Shiga in Japan discovered *B. dysenteriae*. He made plate cultures from the stools and then sought for an organism in these cultures that would be agglutinated with the patient's blood serum. In this way he studied 36 cases and obtained a specific organism in 34. He also showed that the serum of other individuals suffering with the disease sometimes agglutinated the bacillus in question. His work was confirmed in 1900 in the Philippine Islands by Flexner, Strong and Musgrave, who also isolated and described different strains of the organism. Dysentery was produced in a prisoner who voluntarily ingested a Philippine culture and dysentery has since resulted from accidental laboratory infections.

In the United States in 1903 Hiss and Russell isolated an organism from a fatal case of diarrhoea in a child which they designated with the letter Y, while Duval in 1904 reported upon the presence of a lactose fermenting organism in cases of dysentery which later received the designation Sonne type. In 1900 Kruse, who had worked on the bacteriology of dysentery for some years and described other bacteria as the cause, also confirmed in Germany Shiga's discovery of *B. dysenteriae* and also described a lactose fermenting type E of the pseudodysentery bacilli (Sonne type).

Since this time we have gradually come to recognize that the dysentery bacilli comprises a large group of microorganisms which may be separated into a number of types, especially by means of their cultural characteristics, but more importantly by their serological reactions. In recent years many other types have been described in addition to the 4 or 5 classical types already mentioned. However, many of these strains are liable to mutation. Some investigators, especially Busson (1937), believe that under the influence of bacteriophage it is possible to produce secondary types different from the original one. With reference to morphological and cultural variations which have been described, the antigenic structure is of far more importance in defining smooth and rough types than is the appearance of the colonies.

**Morphology and Cultural Characteristics**—The dysentery bacilli occur, as a rule, singly or in pairs, and do not form threads or filaments. They are somewhat plumper than typhoid bacilli. They may be separated from the group of *Coli* bacteria in that none of them produces gas in glucose. The dysentery bacilli, in their cultural characteristics, do not resemble to some extent the typhoid bacillus, but they can be distinguished from it by the fact that they are non-motile, that they have different reactions in sugar media, as well as by their serological relations. The reactions with fermenting sugar media are illustrated in the accompanying tables. The most striking cultural reactions are (1) that none of the dysentery bacilli produce gas in glucose, do

In recent years epidemics have been reported in France Germany Great Britain Russia Southern Rhodesia and the United States. In the United States and northern Europe the disease has not prevailed in severe epidemic form though numerous small outbreaks of moderate size as well as sporadic cases have been reported and studied. The outbreaks have occurred especially in insane asylums orphanages or other public institutions and often in young children. In France Italy and Germany they have been particularly reported in barracks and encampments. Strains of dysentery bacilli have frequently been isolated from cases of so-called summer diarrhoea of infants though in many other instances Morgan's bacillus has been encountered the pathogenic role of which has not been demonstrated. Douglas and Colebrook found it in the faeces of men convalescent from dysentery in Gallipoli. Zinsser and Bayne Jones (1939) point out that strains of the Morgan bacillus are more closely allied to *B. coli* than to the dysentery or typhoid or paratyphoid groups of organisms.

Several small outbreaks of bacillary dysentery have been reported from New York State since 1938 (Gilman Coleman and Leahy). That the disease is not prevalent is evident from the fact that in 1932 only 20 cases of dysentery of all forms were reported from New York City and in 1933 only 89 cases. Whether endemic foci of bacillary dysentery exist in the southern United States as they apparently do in some areas in France (Tours Chalons) has not been carefully studied. Kemp and Haberman (1934) during an epidemic of acute bacillary dysentery in Dallas Texas made cultures from the stools of 58 cases. From 6 of these dysentery bacilli were obtained. Kessel (1936) has observed over a thousand cases in southern California in 2 years. Haven Emerson (1942) has pointed out that among the 7½ million people in New York City that the deaths from Bacillary Dysentery and Diarrhea and Enteritis under five years of age numbered 334 in 1939 250 in 1940 199 in 1941. The death rate from this disease was 11.2 for the United States as a whole in 1940 but has varied widely in different sections of the country being higher in the South Atlantic district with a rate of 17 and in the East South Central 22.4 and in the West South Central 33.6 whereas in the New England district it was 4.7. The percentage of deaths from all causes which were attributed to diarrhea and dysentery were 2.2 among whites and 2.6 among negroes in 1940.

### ETIOLOGY AND EPIDEMIOLOGY

The predisposing causes include bad sanitation and factors liable to lower the resistance of the individual such as exposure to extreme heat or cold errors of diet and drink and fatigue especially during military service. Irritation of the bowel by foreign material of any origin may predispose to dysentery and acclimatization to the tropics is also undoubtedly a factor new arrivals being especially prone to the disease. The presence of any debilitating disease also often predisposes to an attack of dysentery.

**Climate**—The disease most generally occurs in the warm and moist months of the year. However outbreaks are particularly influenced by hygienic conditions and the presence of flies rather than by climatic influences alone. In India as Rogers has pointed out (1930) the minimum prevalence of bacillary dysentery is in the dry, cold weather of January and February. A slight rise occurs in March with an increase of the monthly temperature and a slight fall in the hottest months of May and June but the main rise takes place in the rainy season from late in June to September. On the whole the seasonal distribution corresponded well with periods of fly dissemination. Leddingham's investigations in Mesopotamia made during the War also show that while in general the disease prevails more in the warmer months its incidence is more influenced by the occurrence of the fly season. He found that the first epidemic rise in that area with very low rainfall followed about two weeks after the March commencement of the first fly season. A partial decline of dysentery took place in the very hot months of July and August when the flies disappeared. A second dysentery maximum followed the second fly season in November. Manson Bahr (1939) also notes that the incidence of house flies coincide very closely with epidemics of bacillary dysentery and that the incidence of the disease rises during the rainy season.

fifth type differed from the fourth in that after 24 hours it gave an acid reaction in mannite but this gradually disappeared the medium subsequently becoming alkaline. He regarded this fifth type as an intermediate one between the acid (fermenting) and non acid dysentery bacilli. Andrews and Inman do not mention this type. This classification of Hiss, Lentz and Shiga has been in general use and is generally accepted. However, still other types of dysentery bacilli have since been described and recent investigations carried out during the World War have suggested other classifications.

Duval in 1904, Kruse in 1907 (the Kruse E type) and Sonne in 1915 have all described as a cause of dysentery bacilli which ferment lactose (*B. dys*). Andrews (1918) and it has been suggested that a fifth main group of dysentery bacilli may include the dulcitate fermenting *Bacillus alcalicus* (Glynn and Robinson 1918). Zinsser and Bayne Jones (1939) believe that the evidence that *Bacillus alcalicus* is pathogenic is not convincing. They however include the organism in their last table giving the differential characteristics of dysentery bacilli. The Schmutz bacillus which is differentiated from the Shiga bacillus in this table by the production of indol is classified as a separate organism.

The bacilli of the four classical types (as designated by Hiss, Shiga and Lentz) are not necessarily confined to different geographical regions. The investigations in the Philippine Islands of Flexner, Strong and Ohno have shown that at least 3 of these types of organisms occur there and Amako (1905) in the study of an epidemic in the town of Kobe in which dysentery bacilli were isolated in 526 cases found all of the 4 types of dysentery as well as the fifth type of Shiga. In 6 families in which there were 25 patients he found 2 different types. Such investigations show that even a local epidemic may be caused by different types of the dysentery organism and in large outbreaks it is not unlikely that several types of dysentery bacilli may be encountered.

Shiga points out that the dysentery epidemic in Tokyo previous to 1904 was caused almost entirely by the non acid type. However, Futaki (1904) found that during the epidemic which spread through the capital of Japan in that year in 100 cases studied all were of the acid type and only 2 of the non acid type. In the same year in Manchuria, Chosin and Port Arthur Hata found a greater number of cases infected with the non acid strains.

Ohno (1906) working in the writer's laboratory in the Philippine Islands upon a large number of freely isolated strains believed that the groupings of the different organisms according to the differences in their powers of causing fermentation does not always correspond to that which results from differences observed in agglutinative and bacteriolytic action with specific immune sera.

Many other observers who have sought for the 4 classical types have found them in varying proportions in widely different parts of the world. Thus Ten Broeck and Norbury in 1916 in a study of infectious diarrhoea in infants in Boston found that 76 per cent belonged to the type Hiss, 13 per cent to the Flexner and 10 per cent to the Strong type. Bernstein, Kling and Rosenblatt (1930) in Vienna found the strains Shiga, Flexner, 1 and Strong in 65 per cent of 901 cases of dysentery during and after the War.

De Assis (1934) in a review of bacillary dysentery as it occurs in Brazil states that the organisms isolated in epidemic and endemic bacillary dysentery are (1) the bacillus of Shiga, (2) the bacilli of the group of Flexner concerning the type Strong, 1 and Hiss, (3) the bacillus of Schmutz distinguishable from the Shiga bacillus by its production of

not coagulate litmus milk and that they may be separated into two large groups by their reactions in mannite as was first shown by Martini and Lentz in 1902. The Shiga Kruse Schmitz group do not ferment mannite while the organisms of the Flexner Strong Hiss Russel group and the Kruse Sonne type ferment this sugar. Only the Duval Kruse Sonne type ferments lactose.

The proportion of the different types isolated varies in different epidemics. In some epidemics only 1 or 2 of the types are isolated, while in many endemic areas all of the classical types have been found. In the past few years the Sonne type has been more sought for and found more commonly in the United States. In a study of 300 cases of diarrhoea and dysentery in Virginia McGinnis and his associates 1936 found about 100 due to Flexner types and 50 due to the Sonne type. This organism has also been isolated commonly in Great Britain in the past few years while Wolff (1938) has reported it to be the prevailing form in Sumatra in Europeans.

The serological behavior of the dysentery bacilli is complicated. The organisms of the Shiga group which are non mannite fermenting are generally homogenous but an anti Shiga serum has some agglutinating action on most of the strains of the mannite fermenting organisms (Flexner group). A serum procured with Schmitz's bacillus will also agglutinate some Shiga strains to half its titer. The group of dysentery bacilli (Shigella) contains at least 5 more or less common antigenic components corresponding to the classical strains of dysentery bacilli (as shown by Shiga, Ohno, Morgan, Martini and Lenz and many others). More recently a reclassification of the dysentery bacilli has been attempted in England by Andrewes and Inman (1919), Glynn and Robinson (1918) and in Germany by Sartorius and by Kemper (1932). Some of the strains collected from different laboratories and investigators by Andrewes and Inman apparently have been assumed but not demonstrated to be the cause of dysentery in man nor has any report of their pathogenic action been made. However by the ingestion of cultures of at least 4 of the classical types already described dysentery has been produced in man.

**Differentiation and Distribution of Different Types of Bacilli**—A consideration of the differentiation of the strains of the dysentery group which differ serologically is of some interest and importance from the standpoint of bacteriology but to a less extent in the diagnosis and treatment of the disease.

With reference to distribution of the different strains of bacilli of the dysentery group (Shigella) it may be noted that *Bacillus dysenteriae* (*Shigella dysenteriae*) first isolated by Shiga in 1898 in an epidemic of dysentery in Japan was subsequently shown (first by Drigalski and Lentz) not to ferment mannite. In March 1900 Flexner isolated in Manila from 3 fatal cases of dysentery another strain of dysentery bacilli which was later shown to ferment mannite. In May 1900 Strong also isolated in the Philippines other strains of dysentery bacilli and reported with Musgrave upon a bacteriological and pathological study of 111 cases of dysentery with autopsies 31 of which were of the bacillary dysentery type. At the same time a bacteriological study was made of 71 clinical cases of infection with *Bacillus dysenteriae*. Among the dysentery bacilli isolated from these cases in the Philippine Islands some were subsequently found to ferment mannite (Flexner type) (subsequently described as the V race of Andrewes and Inman 1919), others were found not to ferment mannite (Shiga type) while a third type was found not to ferment mannite but to ferment saccharose [Strong type of Lentz (1902), H type of Ohno (1906) (subsequently described as the W type of Andrewes and Inman 1919)].

In 1900 and 1901 Kruse also isolated dysentery bacilli in Germany and first distinguished culturally between several types. In 1903 Hiss and Russell isolated in the United States another strain of dysentery bacillus which also fermented mannite but which did not ferment maltose. In 1904 Hiss published a classification of the dysentery bacilli based upon fermentation reactions and agglutination tests dividing the organisms described above into 4 main groups and found that strains from many sources could be distributed among these groups. Lentz and Martini (1902, 1913) also separated the dysentery organisms into these same 4 groups.

Shiga in a further study likewise divided the dysentery bacilli by cultural reactions and serum tests into 5 types. The first 4 types agreed entirely with those of Hiss, the

designated by different letters of the alphabet or numbers. It may be necessary to employ a duplicate alphabet to designate all races which have been reported as different or which have been given different letters or numbers. In Germany Sartorius and Reploh 1931 and 1932 have recognized 11 races and Kemper divides them into 6 or 12 races which he designates by the letters A B C D E F G H X D X Y, Y.

**Summary**—This discussion of the question of the differentiation of the dysentery bacilli has been given here in order that the clinician and student may have knowledge and understand the bacteriological studies which have been undertaken in regard to the diagnosis and treatment of the disease. However while such detailed studies are of interest especially to bacteriologists the results to the clinician must be to some extent confusing and moreover they have not been uniform.

A more accurate differentiation of the dysentery bacillus is complicated by the fact that in some of the recent laboratory investigations (1) cultures have not been made and the patient studied in the acute stages of the dysentery as well as in the later stages (2) that in regard to some of the investigations undertaken (particularly those in connection with the World War) the investigators did not take into account a consideration of possible dissociation in the types they described and the types compared in smooth and rough colonies nor were immune sera prepared from all the known pathogenic types and used in the attempted classification of the strains collected (3) the influence that the bacteriophages may have had in initiating a change of type at different stages of the disease was not investigated.

Manson Bahr (1930) points out that the matter has been further complicated by the still finer differentiations suggested by workers on the mannite fermenting group and that the matter cannot yet be considered as having reached finality.

As pointed out Andrewes and Inman state that in their investigations no well known strain of the classical types which had reached them fell into either their X or Z groups. Whether these X or Z strains are capable of producing dysentery in man or are mutations of the classical types is speculative. Boyd (1940) has still further investigated the matter of classification. He states that Andrews's V W and Z races are valid types but that he believes X and Y are not valid types. As has been pointed out in this article the descriptions of the races termed V W and Y correspond to 3 of the classical types and have been repeatedly isolated since 1900 by experienced bacteriologists in different parts of the world. Boyd now proposes to add in place of 2 of Inman's types, 1 of his own isolated in India and termed *B. dysenteriae* Boyd I II III Shiga (1936) has made a plea for the return to the original simple classification.

Species of *Shigella* which have been encountered first in Great Britain and later in the United States are known as the Newcastle type. Peculiarities noted for this organism are that occasionally a slight bubble of gas is produced in dextrose and dulcitol when dissolved in beef extract product dextrose dulcitol and maltose are always fermented to acid and gas. Indol is not formed. The strain is serologically homogeneous and unagglutinated by anti sera prepared against *S. dysenteriae* or *S. paradyenteriae*. Sachs (1943) has reported upon 8 new types of non manitol fermenting bacilli in India and Egypt.

Neter (1942) points out that a number of species now classified as members of Genus *Shigella* have not been adequately investigated and little is known of their cultural characters biochemical actions and antigenic structure.



indol and its serological reactions and (4) the bacillus of Sonne or Castellani Kruse Sonne bacillus

Kemp and Haberman (1934) in the study of 58 cases of bacillary dysentery in Texas found that among 26 cultures of dysentery bacilli obtained 16 were identified as *Shigella paradysenteriae* var Flexner 5 as *Shigella dispar* Andrews and 5 as *Shigella paradysenteriae* var Sonne

In a recent epidemic in Poland studied by Amzel 1935 and his associates all 4 of the original classical strains were encountered

While many strains of dysentery bacilli were studied during the War much of the work was performed in field laboratories and the time obviously was not as propitious as was desirable for deliberate study Other investigations were carried on in European laboratories some upon convalescents after their return from the war area Such studies are not as advantageous in many respects as they were not carried out during epidemics with the cases in the acute stage of dysentery It is an accepted fact that dysentery bacilli frequently cannot be isolated after the first few days of the disease and after the 6th day isolation becomes increasingly difficult Also mutations have been noted in subacute cases

Andrewes and Inman (1919) studied a large number of strains which they rather hurriedly collected from a number of laboratories and investigators However a number of investigators were away from their laboratories on war duty and comparatively few cultures were sent from tropical countries More were apparently isolated from individuals who had served on the western front They divided these organisms into 5 main races which were designated as V W X Y and Z respectively but with sub groups designated as VZ and SX Their V race they state corresponds with most of the strains sent them from a number of European laboratories as Flexner strains However they found no well known strain of the classical types which had reached them fell into either their X or Z groups The so called Y of the Oxford laboratory they classify in their Y group Their Y group corresponds to the typical bacillus of Hiss and Russell while the Strong type corresponds to their W group although the writer sent them no cultures for study They point out that it is of course unlikely that their observations have covered all the serological varieties of the Flexner group Unfortunately they did not study problems relating to dissociation mutation of the dysentery bacilli and the presence of bacteriophage to such mutations or isolate organisms themselves from cases of dysentery

The investigations of Glynn and Robinson (1918) were based upon the study of the excreta of 2360 cases of enteritis received from the eastern Mediterranean area the great majority of whom were convalescent They point out that the work is unavoidably incomplete and regret that they had no opportunity to study acute cases

Their investigations however support the fact shown by Shiga Barber Ten Broeck and more recently emphasized by Martini (1917) and others that mutation of bacteria may complicate the classification of dysentery bacilli when based on cultural changes especially as it seems to affect maltose and saccharose In their classification they include under Flexner Y strains some organisms which fermented saccharose They found 31 Flexner like strains which when first isolated produced acid in saccharose ( Strong type ) Apparently they did not prepare sera of all the classical types of the dysentery bacilli and test the agglutinability of the organisms isolated with all these sera With a Flexner serum they found that only 4 early saccharose fermenters agglutinated out of 19 tested whereas 45 of the 60 strains which were apparently non saccharose fermenters agglutinated with this serum They frequently encountered *Bacillus morgan* and *Bacillus faecalis alcaligenes* but were unable to express themselves regarding the production of dysentery by these organisms Raychman and Weston on the other hand found 3 out of 9 strains of mannite fermenting organisms produced acid in saccharose and agglutinated in high titer

From these and other investigations it seems evident that the group of mannite fermenting or Flexner dysentery bacilli as the group has been termed is very heterogeneous and indeed some observers have pointed out that it is exceedingly difficult from the publications regarding the war studies to obtain an intelligent idea of the relationship of a number of races described by different investigators as new and

Eventually it is hoped a reclassification of the non fermenting manitol organisms will be made including the 5 strains recently described by Sachs (1943) and isolated in India and Egypt

Colonel J S K. Boyd Director of Pathology Middle East Forces in a recent report points out that they have given up examining stools of dysentery cases bacteriologically as they have now all the information they want as to incidence and it does not help the clinician in any way to know what particular organism is responsible in most cases. He believes all the necessary information can be obtained from microscopic examination. This policy has relieved the laboratories considerably and enabled them to devote themselves to more important work.

In practice for purposes of diagnosis and in treatment it is sufficiently satisfactory to separate the important dysentery bacilli into 2 main types with which Shiga 1936 is in conformity

1 *Shigella dysenteriae* (Shiga Kruse type) which produces no acid in mannite media and does not form indol

2 *Shigella paradysenteriae* (*B. Flexneri*) (Flexner Hiss (Y) Strong Boyd and others) which produces acid in mannite media and form indol

The Shiga type is homogeneous in its antigen content and may be differentiated from other types serologically although there is usually some group agglutination with other types. In its growth it forms a potent exotoxin as well as an endotoxin. Intravenous injection of the bacilli or of their toxins into rabbits has sometimes produced a haemorrhagic enteritis followed later by paralysis of the hind legs. An anti serum can be prepared which possesses antitoxic and antibacterial properties and has been reported to be of therapeutic value if given early.

In order that the reader may have in convenient form the older and the more recent classifications of the organisms of the dysentery group of bacilli the following tables are inserted

COMPARATIVE CLASSIFICATION OF THE DYSENTERY BACILLI ACCORDING TO DIFFERENT AUTHORS

Bacillary	Adw adim	Kruse	Leiss Prigg	S	Al
Shigella dysenteriae (Shiga) Cattle dysenteriae	Bacilli Shiga	True dysentery bacilli	Shiga Kruse bacilli	Shiga Kruse bacilli	Group VIII
Shigella boydii (Adw) Widd	Bacilli boydii	Pseudo-dysentery bacilli I & J	Shiga bacilli		
Shigella dysenteriae (Cattle) Widd	Bacilli F & Y	Pseudo-dysentery bacilli			
	V & (?)	B	Flexner bacilli	Group II	
	VZ	A			
	Y	D	Shiga (H S- R sch)	Group I	Group I
	Z	H			Group X
	W & X	CP			Groups II III IV V (?)
	W	G	Shiga bacilli	Group II	
Shigella (Levi) Widd	Bacilli dysenteriae	Pseudo-dysentery bacilli E	Shiga bacilli	Group III	

In a recent circular letter of the British Army Pathology Laboratory Service a reclassification of the manitol fermenting dysentery bacilli has been suggested according to Boyd

CLASSIFICATION BY FLEET COLONEL NEWTON W LARSEN M.C. (1944)

Original name	Andrews & Inman	Boyd's classification
Flexner	V	Flexner I
Hiss Russell	Y	Not a valid race
Stronach	W	Flexner II
—	X	A variant of Z
—	Z	Flexner III
Boyd 103	—	Flexner IV
Boyd 119	—	Flexner V
Newcastle Manchester	—	Flexner VI
Boyd 88	—	
Boyd 170	—	Boyd I
Boyd P283	—	Boyd II
Boyd D1	—	Boyd III
Boyd P274	—	Similar to alkaligenes
Boyd D19	—	Rare occurrence
Boyd P143	—	Rare occurrence

It seems possible that the dysentery bacillus produces both a soluble toxin and in addition an endotoxin which is more closely bound than the former.

More recently Olitsky and Khigler have differentiated definitely between the so-called exotoxin and the endotoxin. They obtained the exotoxin by growing *Bacillus dysenteriae* for 5 days in alkaline egg broth. Their endotoxin was produced by incubating agar cultures in salt solution for 2 days and filtering. The exotoxin in fractions of a cubic centimeter after an incubation time of a few hours to 4 days produced typical paralysis and severe nervous lesions in rabbits. Powerful neutralization of it could be obtained with the serum of horses that had been immunized with it. They succeeded in protecting with the antitoxin horse serum against 400 lethal doses of the poison. The injection of a large dose of the toxin intravenously into rabbits causes a rapid fall in temperature, respiratory embarrassment and a violent diarrhoea which later might contain blood. If the animals lived a sufficient length of time paralysis sometimes occurred in the posterior extremities. Intravenous inoculation sometimes gave rise to intestinal involvement due to the action of the poison by the intestinal mucosa, particularly the caecum and colon. The toxin sometimes caused a coagulation necrosis of the intestinal mucous membrane. A characteristic action of the exotoxin of *Bacillus dysenteriae* is its effect upon the medulla and spinal cord in rabbits where it may produce lesions not unlike those seen in encephalitis and lead poisoning.

The bacteriocidal reaction of dysentery immune serum may be easily demonstrated *in vitro* as was first demonstrated by Shiga and by the intraperitoneal method of Pfeiffer by Kruse. However the production of Pfeiffer's phenomena in the abdominal cavity of the guinea pig is frequently not satisfactory.

### EPIDEMIOLOGY

**Diffusion**—The occurrence and distribution of the disease as pointed out is often influenced more by hygienic than by geographical conditions. In this respect as well as in its mode of communication it resembles typhoid fever. The dysentery bacilli are usually ingested with food or drink to which they have gained access directly or indirectly from the faeces of cases of bacillary dysentery, sometimes from carriers of the bacilli. In lunatic asylums direct contagion may be common and the latrines a direct source of infection. In a disease where the movements are frequent and fluid or in milder cases not under treatment in hospitals the diffusion of the dysentery organism in infectious material is much more likely to occur than in typhoid fever. Thus transmission from man to man by hands and indirect contamination of food or water is possible during epidemics.

Stitt has pointed out that there is probably no disease with the possible exception of cholera where those attending a patient are so liable to have their hands contaminated with infectious material.

The great frequency of the stools in acute stages and the tendency of the mucilaginous mucoid mass to become smeared over the buttocks and clothing of the patient make it onerous for an attendant to carry out methods of personal protection. In a family living under unsanitary conditions where the mother may have to care for a sick child and prepare food for the other children and herself the opportunities for the spread of the infection in the family are great. In military barracks as well as in other institutions where large numbers make use of the same water-closet accommodations the chances of contamination of the seat by a patient responding to the frequent and imperative demands for evacuation are most probable with subsequent transference of the infectious material to others. Bacillary dysentery is peculiarly an institutional disease and tends to spread in jails, orphan asylums and the like. However the dysen-

An organism which resembles the Shiga type in its inability to ferment mannite but which produces indol and ferments rhamnose is known as the Schmitz bacillus (*S. ambigua*).

The group which ferments mannite is composed of a number of types—*S. paradysenteriae* types Flexner, Hiss, Strong and others which are antigenically heterogeneous, *S. sonnei*, *S. dispar* etc. They produce an endotoxin but many strains do not produce the soluble exotoxin which is characteristic of the Shiga bacillus. Clinically the toxæmia in dysenteries due to these types is usually less than that in the Shiga type.

In addition there are other atypical types such as the Sonne bacillus (*S. sonnei*) which produces acid in mannite and also a slow fermentation of lactose and forms no indol. This represents a distinct serological type and has caused epidemics of dysentery in the United States and in Europe.

*S. dispar* resembles *S. sonnei* except that it (usually) ferments xylose and forms indol. Serologically it constitutes a heterogeneous group. It is probably non-pathogenic.

Dysentery bacilli may be differentiated provisionally by the following cultural reactions regarding the fermentation of sugars.

Shigella	Dextrose	Mannitol	Maltose	Sucrose	Indol
Dysenteriae (Shiga)	+	o	o	o	o
Ambigua (Schmitz)	+	o	o	o	+
Paradysenteriae					
Hiss type (Y)	+	+	o	o	+
Flexner type	+	+	+	o	+
Strong type	+	+	o	+	+
Sonnei	+	+	+	+	o
Dispar	+	+	+	+	+

Recent work has shown that the types of *S. paradysenteriae* may be variable in their fermentative reactions and that groups based on these properties may not always correspond to those based on serological reactions. The latter classification is therefore preferable. For the production of therapeutic sera a number of strains of different antigenic properties both mannite and non fermenting mannite strains should be used.

**Toxins**—Dysentery is probably a true toxæmia its symptoms being referable almost entirely to the absorption of the toxins of the bacillus from the intestine. Experiments carried out chiefly upon rabbits have showed that even small doses of cultures of the dysentery bacilli administered intravenously or subcutaneously produced death in a very short time.

The earlier investigations by Conrad, Neiser and Shiga, Vallard, Dopter, Flexner and Sweet demonstrated that the toxin was chiefly an endotoxin. Later however Todd, Kraus and Doerr, Rosenthal, Kolle, Heller and Neufeld showed that the dysentery bacillus may produce a strong soluble toxin. It seems evident that the dysentery toxin while not identical in action with the true soluble toxin of the diphtheria bacillus nevertheless resembles this toxin more than it does the toxin of either the typhoid bacillus or cholera spirillum.

As was first shown by Todd the antitoxic serum when added to solutions of toxin after half an hour neutralizes the poison in the test tube and upon the subsequent injection of the mixture into rabbits no toxic action is observed. The serum also followed up to a certain extent Ehrlich's law of multiples. Kraus and Doerr have also produced specific antitoxins with the toxin.

Bahr reported that from his experience the great majority of carriers even when apparently healthy are still suffering from ulceration of the intestinal mucosa and that wherever sigmoidoscopy has been carefully performed ulceration or inflammation of the lower sigmoid or of the rectal ampulla have been revealed. The percentage of carriers discovered in different localities has varied greatly. During the War Verzar in 417 cases of convalescing dysentery in Germany discovered 77 carriers while Fletcher and McKinnon in convalescent English troops found some 72 carriers in 935 cases. Sergeant discovered 13 chronic carriers among a group of 67 pilgrims who had recently returned from Mecca. Shiga has particularly emphasized the fact that it is through carriers that infection survives during the inter-epidemic periods in Japan. R. C. Connor and Bates believe that the spread of the disease in Panama is especially if not wholly due to carriers. However Boyd (1940) doubts that carriers are instrumental in the spread of the disease in India.

Duval and Shorer have found dysentery bacilli in the dejecta of healthy children in connection with the study of summer diarrhoea of infants and Martha Wollstein has isolated dysentery bacilli from the intestine at autopsy of children who had not suffered with symptoms of dysentery immediately or shortly before death.

**Animals**—Bowman in 1910 found in two of our laboratory monkeys in the Philippines spontaneous bacillary dysentery. Ravaut and Dopter also isolated the organism from monkeys in captivity in Paris and Scott in one instance from a gorilla. Dogs have also been found infected in a few instances as noted recently by Dold in China but it is not believed that the disease is commonly spread by animals.

**Distribution**—The dysentery bacilli are present in the intestinal tract and in a few instances have been found in the mesentery glands but they do not generally invade the blood stream or appear in the urine though especially in earlier years a few cases were reported in which dysentery bacilli had been isolated from the blood, urine and spleen. As the organism does not generally invade the blood stream we do not find it in the urine (except possibly very exceptionally) so that to a certain extent the dysentery bacillus carrier is less dangerous than the typhoid one. Nevertheless infection of the urinary tract may exceptionally occur. Neter (1937) has reported 3 cases of infection of the urinary tract caused by *Bacillus dysenteriae* and has reviewed 14 cases of dysentery bacillus infections of the urinary system collected from the literature.

The urine specimens from his 3 cases revealed the presence of dysentery bacilli. All were females 2 of them children and one an adult in pregnancy. The patients did not present a history of dysentery or show any clinical evidence of intestinal involvement. At least 2 of the 3 patients appeared to be a carrier of dysentery bacilli and the dysentery bacillus was demonstrated in the intestinal tract even 4 weeks after the child had recovered from the acute dysentery bacillus cystitis. Dysentery bacilli were never recovered from the faeces in the second case. All 3 patients recovered within a few weeks. Both children were treated with mandelic acid the third patient with methanamine. The patients showed agglutinins in their serum with the dysentery organism.

It is possible that infectious material may sometimes be disseminated in moist dust and thus contaminate food but this is evidently not a common means of infection or spread.

tery bacilli are frequently not present in very large numbers in the dejecta except during the acute stages and the disease is probably not widely disseminated in this manner

Fly transmission is usually of much greater importance and is likely to occur in the tropics especially among the inhabitants of rural villages or troops in camp where open latrines are employed, and kitchens food and mess tables etc are unscreened. It has been well recognized that dysentery bacilli will live for several days in the intestine of the fly and be passed in the dejecta. Manson Bahr demonstrated *Bacillus dysenteriae* in the intestinal tract of the house fly 5 days after its ingestion. Taylor in Salonica and others have also isolated dysentery bacilli from flies, Taylor finding that the organisms diminished rapidly in the fly after 24 hours from the time of the original infection.

The *Bacillus dysenteriae* may remain alive also for considerable time in moist soil, and the local water supplies may be contaminated with faecal material which has given rise to sharp outbreaks. In the Philippine Islands in 1899-1900 probably numerous infections occurred from men filling their canteens (contrary to instructions) with infected water collected on the march or about temporary camps. Evidence has also been given of water acting as the medium of infection in the Malay States by Fletcher and Jeps by Manson Bahr in Fiji, and by Dudgeon in Salonica during the war. It has been demonstrated that the dysentery bacilli may survive in drinking water for over 3 weeks. However, they are readily destroyed by the direct action of the sun's rays.

**Carriers**—The disease may also be spread by carriers especially those who are convalescent from the disease as the investigations of Conrad Shiga, and many others have demonstrated. Carriers among cooks and handlers of food may obviously be especially dangerous. It is now thought that the striking prevalence of the disease in insane asylums is associated with the difficulty of making such patients observe the proper care of their hands as well as their persons.

Friedmann has noted an outbreak of dysentery due to the Shiga type of bacillus which was instituted by a soldier returning to the barracks from a furlough. There resulted 86 cases in the man's regiment of which 49 belonged to his own squadron. The spread of the disease was traced to the latrines. The epidemic was suppressed by the enforcement of the most rigid rules of cleanliness especially as regarding washing of the hands after leaving the latrines.

The stools of the convalescents were examined and no man was discharged from the hospital unless his stools were negative for dysentery bacilli upon 3 successive tests in 14 days. Isolation of the bacilli from convalescents was obtained in 40 patients only for periods under 14 days while with 27 others such carrying of bacilli lasted from 2 weeks to 1 month.

The carrier state in bacillary dysentery does not as a rule persist for any great length of time though a few exceptions have been reported. Perry found that the carrier state frequently persisted from 4 to 6 months and at the end of a year nearly 4 per cent were still carrying dysentery bacilli of the Shiga type but in the case of infections with the Flexner type the percentage was 7. Hudson reported a case of Flexner infection in which the bacillus could be demonstrated for 3½ years. Manson

Its presence may be demonstrated in either fluid or solid cultures. Inoculation of a suitable broth culture causes a partial or complete clearing of the turbidity in from 4 to 24 hours. The potency of the bacteriophage preparation can be measured by adding varying amounts to a fresh culture. In some cases as little as one part of the filtrate in a billion parts of culture will effect lysis. If transplants of such a mixture are made to an agar plate shortly after inoculation a surface growth is obtained which is pitted with clear glassy areas or plaques. These plaques may occur in the center or around the edges of discrete colonies giving the culture a moth eaten appearance. Smears from these areas show only an amorphous debris. When lysis becomes complete no growth is obtained in subcultures. D Herelle refers to these plaques as colonies of bacteriophage.

The growth of bacteriophage in a culture frequently results in the development of variant types of the culture which may be enhanced or diminished in virulence. Some forms may be changed to R forms and mucoid types frequently develop.

*Mechanism of Lysis*—Under the microscope the affected bacteria can be seen to swell to a relatively great size and burst suddenly. D Herelle believes that the phenomenon is due to multiplication of the bacteriophage within the cell. Brousenbrenner has suggested that the decomposition of the proteins of the cells by some ferment or enzyme may raise the osmotic pressure and cause water to be absorbed into the cell.

*Stability*—Bacteriophage may withstand heating to 75 C. for  $\frac{1}{2}$  hour and resists drying for some time. According to D Herelle its resistance in general is greater than that of vegetative bacteria and less than that of sporebearers.

*Specificity*—Bacteriophage is generally (though not always) specific for certain species of bacteria. It may act upon closely related species. Sometimes its activity is limited to a certain strain of a species. Adaptation to other organisms may occur to some extent. D Herelle believes however that there is only one very adaptable bacteriophage while others believe that there are distinct types. It has been found to be antigenic. Since the bacteriophage cannot be obtained free from lysed bacterial bodies antisera contain also ordinary bacterial antibodies. After absorption of such sera with the bacteria themselves however a substance remains which will inhibit the action of the homologous bacteriophage. This antibody is said to be specific for the bacteriophage used in its production irrespective of the bacterial substrate upon which the bacteriophage was propagated. Stutt and Clough (1938).

*Use in Therapy*—D Herelle believes that bacteriophage action plays a significant part in recovery from infection and in the rise and fall of epidemics. He claims to treat successfully such infections as dysentery typhoid plague cholera etc. with bacteriophage preparations. The results have not been confirmed by others. Such solutions are very antigenic by reason of the lysed bacteria which they contain and this fact must be remembered in evaluating the results obtained.

A number of authors however report good results from its use locally and parenterally in local and septic infections and also in colon bacillus infections of the urinary tract. Krestownikowa and Gubnik have demonstrated that when bacteriophage preparations are injected parenterally the lytic agent is found in all the tissues within a few minutes but disappears within 6 or 8 hours.

Bacteriophage as was mentioned was first discovered in relation to *Bacillus dysenteriae* (Shiga). It was later found that the dysentery phage



**Bacteriophage**—In certain dysentery epidemics none of the types of dysentery bacilli which have been described could be found. This was sometimes supposed to be due to scarcity of dysentery bacilli present in the dejecta. It has been generally recognized that the dysentery bacilli are most prevalent in the dejecta only in the early and very acute stages of the disease. Later D Herelle (1917) suggested an explanation for these results and it was in connection with the study of dysentery that the discovery of his bacteriophage was made. He made the interesting observation that if the dysenteric dejecta were diluted with bouillon and then filtered, and small quantities of the filtrate were added to bouillon cultures of the dysentery bacilli the bacilli were dissolved. It was later assumed that the action of the lytic agent was due to an invisible filterable virus which was able to multiply only in the presence of living bacteria. He found that this 'bacteriophage' appeared during the first week of the disease and usually persisted into the third or fourth week.

Other investigations showed that bacteriophage is a filterable yet particulate substance which causes lysis, or dissolution of susceptible bacteria. It increases in quantity in the process and can be transmitted indefinitely from one culture to another.

**Discovery and Nature of Bacteriophage**—Twort in 1915 while working on the filterable viruses observed a glassy degeneration of certain colonies of cocci in which no intact organisms were demonstrable in smears. Inoculation from these areas into normal colonies produced similar areas. This lytic agent was filterable and could be transmitted serially in cultures. In 1917 d Herelle discovered that sterile filtrates from the faeces of a case of Shiga bacillus dysentery contained a substance which would after a preliminary period of incubation (4-24 hrs) inhibit and finally dissolve the organisms in an actively growing culture of the Shiga bacillus. He also found that filtrates from this lysed culture acted similarly on a fresh culture even in minute amounts and that the lytic principle could be propagated indefinitely in Shiga bacillus cultures.

The nature of this lytic substance is still disputed. D Herelle who has been the most active investigator of the problem is convinced that it is a minute organism living as a parasite on susceptible bacteria and named it *Protobios bacteriophagus*. Many other investigators however believe that the phenomenon is due to autolytic ferments derived from the bacteria themselves. Bordet and his associates postulate the theory that a mutational change which he terms a hereditary nutritional vitiation occurs in the bacteria. These variant strains then develop the property of producing active lytic substances which in turn affect other sensitive bacteria. In order to explain the development of lysogenic strains (which contain the lytic principle but are themselves resistant) he further assumes that such strains continue to produce the lytic agent and transmit this property to other generations yet are themselves able to resist the lytic action. Hadley has made the suggestion that the bacteriophage is perhaps a filterable phase in the life cycle of the bacteria.

**Demonstration in Cultures**—Bacteriophage can be obtained easily from faeces or sewage. It is often present in water and soil and has been isolated from pus infected urine and other substances. It is sometimes present in laboratory cultures in a latent or even in an active form. After isolation by filtration its potency can be increased by repeated inoculation into susceptible young cultures. Old or dead cultures are not affected. It is never demonstrable except in conjunction with actively growing young cultures.

more diffuse (bright red) haemorrhages with irregular margins measuring from 2 to 4 mm or in other instances a centimeter or more in diameter. The solitary follicles are generally swollen and raised and of a bright red color. Scattered about them may be small red sharply circumscribed purpuric like spots. Occasionally the background of the intestine may be described as though covered with a bright red eruption but with darker red haemorrhagic areas scattered over this background. In the very acute cases no definite ulceration takes place but only this more or less superficial coagulation necrosis of the mucosa.

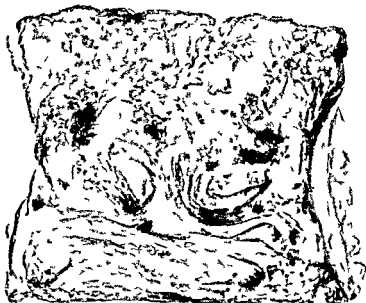


FIG 139—A tabillidytrophy. Death on the 4th day of the disease

On opening the *small intestine* the mucus surface throughout may be normal but in about one third of the acute cases the lower 10 or 15 centimeters of the ileum is involved. When this is so the intestine presents in general the same reddened haemorrhagic appearance and is covered with a necrotic mucus layer as in the large bowel. However there is not the oedema and thickening of the intestinal walls as observed in the large intestine. Peyer's patches may be only moderately swollen or in some instances appear not involved. The solitary follicles are often raised and haemorrhagic. The process is never so well marked in the ileum as in the large intestine.

When death occurs in the *subacute form* the mucosa of the intestine has lost its bright red look. The solitary follicles may be more swollen haemorrhagic and of a dark red color. The surface of the bowel is some

is specific for both mannite and non mannite fermenting strains and is eliminated in the intestinal canal of patients recovering from bacillary dysentery. Evidence was also obtained that different bacteriophages were developed in the intestine according to the invading organism and Milles 1937 has emphasized the importance of identification of the different types of dysentery bacilli by means of the specific bacteriophage. By such means he has reported that he has made a diagnosis in 95 per cent of cases in 24 hours of the types Shiga, Flexner, Hiss and Strong.

It has been observed that during the course of the dysentery there appear to be fluctuations in the virulence of the bacteriophage obtained from one individual case and also in the resistance of the bacteria and it has been maintained that the beginning of an improvement in the patient's condition coincides with the time that the virulence of the bacteriophage excreted in the stools dominates the resistance of the dysentery bacillus. D Herelle believed that the conditions reproduced in the living body are the same as can be observed in the test tube. He found that in fatal cases of bacillary dysentery at no time during the course of the infection did the intestinal bacteriophage show any activity on the dysentery bacillus either for a laboratory strain or for those strains isolated in stools from the patients. The belief was held that in an epidemic cases of diarrhoea may be in reality cases of aborted bacillary dysentery due to the rapidity with which the intestinal bacteriophage adapted itself against the invading organism.

Reference to the use of bacteriophage as a therapeutic agent will be discussed under treatment.

#### PATHOLOGY

While the microorganism of cholera is one which affects especially the epithelium of the small intestine the dysentery bacilli affect especially the epithelium of the large intestine.

*The Acute Form*—The pathology varies considerably according to the virulence of the infecting organism and the susceptibility of the individual. In the cases which have been severe and acute on opening the abdomen the submucous and muscular coats of the large intestine are usually oedematous and swollen the blood vessels of the submucosa injected and in places there are diffuse haemorrhages. In some instances fine flocculi of fibrin are present on the peritoneal surface indicating a lymphoid peritonitis. On opening the large intestine usually its whole length is involved from the caecum to the anus. In acute cases the mucosa is covered with a superficial layer of mucus and necrotic material. This may extend for a distance of 10 or 12 cm into the ileum. This necrotic layer consists of mucus, red blood corpuscles, polymorphonuclear leucocytes, epithelial cells and many large swollen macrophages some containing red blood corpuscles. Many bacteria are also present. If one brushes this mucus layer lightly aside the bright red injected appearance of the intestinal wall becomes more plainly visible (Fig 139). Dotted here and there throughout there frequently are small sharply circumscribed or

sometimes diffuent. The kidneys are frequently congested and both kidneys and liver may show parenchymatous changes. In a few cases central necrosis of the suprarenal glands has been observed.

**Advanced Lesions**—In other instances in which the patient has succumbed later in the disease the entire large intestine may be greyish red looking like lustreless red velvet. Still later changes consist in the devel-



FIG. 41.—Mucous membrane in bacillary dysentery. Collected from a patient who died.

opment of irregular islands composed of greyish membrane surrounded by the red swollen congested gut. The solitary glands are usually swollen and may soften and ulcerate having the submucosa as a base. Ulceration in bacillary dysentery is superficial rather than deep as is usual with amoebic dysentery. The ulcers of bacillary dysentery more often involve the free folds of the intestine and extend transversely while amoebic ulcers run longitudinally. The intervening mucosa is usually unaffected in amoebic ulcerations while in bacillary ones it usually is inflamed.

The ulcers of bacillary dysentery are not undermined. In the later stages of the disease there is no longer evidence of an acute process about

times uneven due especially to the irregular thickening of the mucosa. The necrotic layer of mucus is much less profuse than in the acute stage. There may be still no definite ulcers but superficial erosions are observed and white, grey, or greenish patches covered with diphtheritic or false membrane may be present, Fig 140. In removing this false membrane, portions of the mucosa may come away with it. In the most

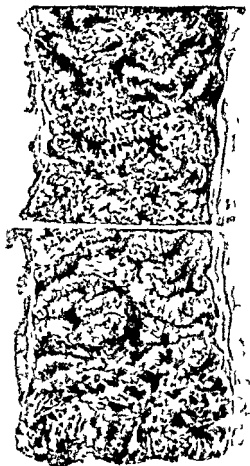


FIG 140 —Bacillary dysentery. Death on 10th day of disease revealing ulceration, necrosis and diphtheritic like patches on the surface of the mucous membrane.

severe cases almost the whole intestine may show this process. In cases which have died later in the disease the mucosa may take on a mammillated appearance.

**Other Organs** —The mesocolic glands, especially those adjacent to the large intestine, are usually swollen and frequently haemorrhagic. In those who have died early in the disease there is no emaciation. The right side of the heart is frequently engorged and the spleen dark red and

in their production. In some of the cases which have become chronic the pathological condition resembles that which has been described for idiopathic ulcerative colitis.

Bargen (1935) of the Mayo Clinic has believed this condition due especially to invasion of the lesions with a specific diplostreptococcus.

Paulson (1933) and Crohn (1936) have carefully studied the etiology and treatment of this idiopathic ulcerative colitis which they point out has been so frequently observed in our hospitals. Undoubtedly streptococci play an important role in these ulcerative conditions, many of which result primarily from amoebic or bacillary infection. A specific diplostreptococcus, however, cannot be regarded as the primary infecting agent of this group.

Mackie, in a study of 83 consecutive cases of chronic ulcerative colitis in New York, found that 42 per cent presented cultural or serological evidence of bacillary dysentery and dysentery bacilli were recovered in 20.4 per cent. In the cases of long standing in which the intestine is chronically thickened and sclerosed the primary infectious agent may already have disappeared.

**Sigmoidal Lesions**—It is difficult to determine the earliest lesions of mild bacillary dysentery cases since patients do not usually succumb to mild infections. Manson Bahr (1939) has made extensive and careful studies with the sigmoidoscope and he and Biggam (1930) have described what they regarded as the earlier lesions. Manson Bahr thought they appeared to originate in the lymphoid follicles. These becoming infected give rise later to superficial "snail track" ulcers which travel across the bowel spreading on the edges of the transverse folds of the mucosa. In addition there is a catarrhal involvement of the mucous membrane and the secretion of much viscous mucus.

**Complicating Lesions**—Dew and Fairley (1931) in a study of 259 acute fatal cases often observed myocarditis and pericarditis as complications. In one instance a splenic abscess was encountered from which a Flexner strain was isolated.

Remlinger and Dumas have described an acute suprarenal syndrome in 4 per cent of their cases. Diagnosis was confirmed at autopsy by finding hypertrophy of the adrenal. The syndrome appeared in mild as well as in severe cases. Histologically the suprarenals revealed congestion and in some instances diffuse coagulation necrosis affecting both the cytoplasm and the nuclei of the cells.

In chronic dysentery Manson Bahr in a series of some 300 autopsies performed in Egypt found 3 instances of antemortem perforation of the transverse colon with general peritonitis. Such a condition while not uncommon in amoebic dysentery has not been reported before in bacillary dysentery. He has also observed a pathological condition which he regarded as a direct sequel to chronic bacillary dysentery consisting of the formation of tapioca-like mucus retention cysts varying in size from a hemp seed to that of a cherry which were distributed unequally through the large intestine causing excrescences on the peritoneal surface. On incision a clear jelly-like mucus could be expressed. The retained material was often infected with dysentery bacilli and with *Bacillus coli* and might ultimately result in abscesses forming with the bowel wall.

the edges of the ulcers. Frequently their borders and patches of the mucous membrane about them, are greyish or greenish, rather than red. Other bacteria particularly the streptococcus complicates such lesions which may then extend in depth and become gangrenous. In the patients who survive the disease for long periods there is more or less wasting and in some emaciation may be marked.

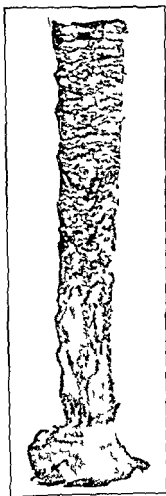


FIG 142—Ileum and ileocaecal valve. The mucosa is swollen and necrotic and covered with a granular deposit of fibrin. (Courtesy of Fletcher and Jepps.)

**Histology**—Microscopically in sections of the intestine from acute cases there may be noted marked congestion of the blood vessels of the mucosa and submucosa, with dilated lymph spaces full of polymorphonuclear cells and endothelial phagocytes the latter often containing red blood corpuscles. In the mucosa, we find an outpouring of pus cells which are entangled along with the glandular structures of the mucosa in a fibrinous exudate resulting in necrosis of the mucosa (coagulation necrosis).

In many instances the crypts of Lieberkuhn's follicles are destroyed. The inflammatory process is usually very intense in the lymphoid follicles.

**Location of Lesions**—Virchow noted the greater intensity of the intestinal lesions in the region of the rectum sigmoid flexure and ileocaecal valve. In India Rogers found that in the chronic cases they were limited especially to the lower portions of the large gut and rarely extended above the descending colon. Willmore and Savage have noted at autopsy cases in which there was a large granulating surface over the whole large intestine in cases which clinically had apparently recovered but in which the convalescence has been greatly prolonged before death. In chronic bacillary dysentery changes in the colon sometimes may be demonstrable by means of X-ray pictures, but as a rule the plates do not give definite information.

**Secondary Lesions**—The necrosis in the earlier cases does not as a rule extend much deeper than the muscularis mucosae. At the base of this coat and through it there is frequently much inflammatory cellular reaction and deposition of fibrin. The ulcerations however, may extend deeper in the chronic cases, where other bacteria are present and concerned

tendency to mental disturbance. The mind however is usually clear. Fever of moderate degree is not uncommon.

In the majority of the severe cases in the tropics the onset is acute with colicky pains, diarrhoea and tenesmus. Within 24 to 48 hours the stools usually consist only of bloody mucus. There may be from 20 to 40 or more small movements within 24 hours. Vomiting is not infrequent. The temperature rises to 101 to 104 or higher and maniacal symptoms may be present but in other cases there may be sudden collapse and the temperature subnormal from the onset. The abdomen at first may be swollen, the intestine distended and later shrunken. There is usually definite tenderness along the colon. The appetite is lacking, the patients usually refuse food as it increases the dysentery and they frequently rapidly sink and die. In the acute cases the stool may be almost pure blood with only an admixture of mucus. Vesical tenesmus may also be present and the urine may be diminished in amount.

The toxic effect on the heart may be apparent so that the pulse tends to become accelerated and weak. The blood changes are not constant. There may be a moderate leucocytosis with increased polymorphonuclear percentage instead of a large mononuclear increase as often found in amoebic dysentery. At times however the lymphocytes may show the greatest relative increase. In rapidly fatal cases the number of leucocytes may not be increased.

In a series of blood counts at the Peking Union Medical College the average counts in 90 uncomplicated cases were from 10,000 to 12,000 while in 14 acute cases of amoebic dysentery the average count was in the neighborhood of 15,000. In a number of the mild cases of bacillary dysentery the white cell count is not above normal. Agglutinins for the dysentery bacillus may appear in the blood after from 6 to 10 days of the onset of the disease hence sero-diagnosis is of no value except in the later stages.

The agglutination test is on the whole unreliable in bacillary dysentery. In some cases the serum may show an agglutination in a dilution of 1:50. In other cases there may be no agglutination in dilutions of 1:10 or 1:20. Normal serum will sometimes agglutinate the dysentery bacillus in such dilutions. The agglutination test is more satisfactory than the bacteriolytic reaction for differentiating the different strains of dysentery bacilli. The use of the test for diagnosis is described later.

In the chronic forms lasting over months the patient usually continues to have diarrhoea and passes loose stools containing more or less mucus. This condition may last for years and end in death from exhaustion or the symptoms may slowly disappear. In such cases caecal papillomata or polypi sometimes appear which have been described in idiopathic colitis.

**Collapse Types**—In the most severe types of dysentery we may have an abrupt onset with rigors and vomiting and high fever. This fever gives way to subnormal temperature and the patient shows signs of collapse and rarely such a case may die without having passed dysenteric stools. The abdomen is rigid and very tender on palpation.

**Enterodysentery**—In those cases where the process extends to the lower portion of the small intestine the general symptoms may be much



He believed that these cysts form through proliferation of the mucous membrane beneath the muscularis and that they may explain the occurrence of mucous colitis so frequently a sequel of bacillary dysentery. Fletcher and Jepps in the Malay States also describe a similar pathological condition in chronic cases of dysentery and in carriers. In cases of chronic infection polypoid growths sometimes result.

An important sequela of severe infections is stenosis of the colon. The large intestine may in some cases become a narrow tube adhesions to the surrounding organs may form and painful peristalsis may result.

**Mixed Infections**—The lesions in the intestine of chronic bacillary dysentery may constitute foci for a general invasion by streptococci or of strains of *Bacillus coli*. The writer observed such terminal infections in the Philippines in earlier years and Manson Bahr and Enright reported 9 cases clinically, and with post mortem examinations in which there was pyaemia, the organism being isolated from the blood and urine. In some cases a variety of colon bacillus was isolated in pure culture from abscesses in the cortex of the kidneys.

**Double infection with amoebic and bacillary dysentery** has been frequently reported in natives, but is not so common in the white race. An unusually high percentage of infection in natives has been reported by Fletcher and Jeps. *Bacillus dysenteriae* having been isolated in 27 of 198 cases of amoebic dysentery. Bacillary dysentery also not uncommonly occurs as a terminal infection in visceral leishmaniasis (kala azar).

### SYMPTOMATOLOGY

Bacillary dysentery usually runs an acute course, rarely relapsing but sometimes going on to a chronic condition. The period of incubation is usually from 2 to 7 days although accidental infection with bacilli in the laboratory has given an incubation period approximating 24 hours. Periods of incubation longer than a week can perhaps be explained as for cholera such cases being in those who are healthy carriers but by reason of some gastro intestinal upset the quiescent bacilli take on pathogenic activity.

In temperate climates and in particular when the infecting organism is a Flexner type (non mannite fermenting and not of a virulent strain) the case may be mild in character with a gradual onset of the intestinal symptoms consisting of a watery diarrhoea associated with colicky pains and anorexia. The stools soon become more scanty in amount frequent in number and associated with straining. This is followed by mucus stools more or less tinged with blood. The temperature is normal or but slightly elevated and the patient does not seem ill.

In the tropics and in temperate climates where a virulent strain of the Shiga bacillus is the infecting organism the onset is usually rather sudden with malaise abdominal pain and a diarrhoea which only temporarily relieves such pain. This initial diarrhoea is soon followed by the characteristic dysentery stool and is accompanied by pain which tends to centre about the umbilicus and to become continuous. There is usually loss of appetite and slight nausea and the patient may at times show a slight

been recognized as dysentery may have been caused by dysentery bacilli and suggests agglutination studies in arthritis cases where no distinct history of dysentery is obtainable. Cope in Salonica has also noted an ankylosing type of arthritis with periarticular thickening resembling true rheumatoid arthritis. In addition to the arthritis there may be neuritis which in severe cases may go on to muscular atrophy.

Parotitis due to secondary infection perhaps through the mouth and throat has been frequently noted as a complication. Rarely conjunctivitis and iridocyclitis may occur. Subnormal temperature may follow severe attacks.

In some epidemics of dysentery *gangrenous manifestations* have been common. This is a very fatal type that is recognized by the passage of dark brown serous discharges containing ashy grey to black sloughs or even tubules of gangrenous mucosa; the stool having a putrid odor. The general symptoms are pronounced, there being a dry glazed tongue and low muttering delirium with a thready pulse. The condition resembles the typhoid state.

**Chronic Cases**—It has been usual to consider bacillary dysentery generally as a self-limited disease running on to convalescence within 10 days to 2 weeks. Rogers has called attention to the importance of bearing in mind a chronic condition especially in natives as well as an acute one. In these chronic cases the ulcerations are usually located in the descending colon, sigmoid flexure or rectum and give rise to frequent stools containing blood and mucus and causing a progressive loss of strength and weight. There is marked digestive disorder and the patient becomes weak, anaemic and neurasthenic. In such cases stenosis of the large intestine may result and atony of the large bowel with post-dysentery constipation. Manson Bahr and others have observed a post-dysentery form of dyspepsia in which achlorhydria and hypochlorhydria have been found.

Lobar or bronchial pneumonia is a not infrequent terminal event in the chronic form of dysentery.

### SONNE DYSENTERY

The Sonne bacillus was first reported by Kruse and his associates who described it as a lactose fermenting or E race of pseudo-dysentery bacilli. In 1904 a lactose fermenting organism was found by Duval in the United States and in 1915 Sonne gave a detailed description of it during an outbreak of dysentery in Copenhagen. Subsequently it was found especially in England, Egypt, Australia, Brazil and since 1930 in a number of outbreaks in the United States. Several small epidemics in New York State and elsewhere have been reported by Gilbert and Coleman (1929) and Leahy (1931). In Japan it has been said to have caused acute dysentery in children which is known under the name of *shirushi*.

The organism is non-motile and resembles in morphological characteristics the dysentery bacilli. On agar forms of colonies have been observed, one round and smooth and the other flat and irregular. The different colonies may vary in agglutination; single smooth colonies giving different reactions from rough ones. On lactose litmus agar the colonies are at first bluish and later reddish. The litmus milk remains unchanged at first but lactic acid is produced and it gives a negative methyl red reaction. On McConkey's medium the colonies frequently show a small central point of acidity on the somewhat opaque background. Mutations of the organism have been

more severe although sometimes the tenesmus is less and the stools less frequent and more voluminous. They contain much blood and mucus mixed with faeculent material. Shiga employed the term 'entero dysentery' for such cases.

In severe cases with more typical dysentery or colo dysentery (as designated by Shiga) as the disease progresses the stools may change from the muco purulent mass to a serous discharge which is very rich in albumin and of an albuminous odor. In such cases emaciation of the patients is very rapid. They may show signs of collapse with cold clammy skin and the clinical picture one associates with cholera. It has been suggested that such cases may be due to action of the dysentery toxins on the adrenal. The serous fluid may contain the flesh like particles which the French liken to gut scrapings. Organisms of the *Salmonella* group are sometimes present in such cases and not dysentery bacilli.

**Complications**—Persistent temperature often indicates complications. There may be derangement of the nervous system mania or temporary paralysis of certain groups of muscles. Delirium may occur toward the close. Inflammation of the joints and tendon sheaths not infrequently occurs.

*Arthritis* may be frequent in one epidemic and not observed in another. The knee joint is the one most commonly involved less commonly the ankles. In a series of cases studied by Graham both joints were attacked in 16 cases and one in 15. The ankles and elbows come next in order of frequency. In some cases the arthritis appears late in the course of the attack. Klein reported 8 cases out of a series of 973 in France in which the knee joint was involved in every case and the onset occurred on the average on the 20th day after the infection. Other observers have reported this complication as occurring from the 6th to 23rd day, while Cope (1920) in Mesopotamia who observed joint trouble in 1 to 2 per cent of bacillary dysentery cases found that this complication developed up to 3 months after the primary attack.

In cases which have been aspirated the fluid was reported as straw colored slightly viscid and usually sterile on culture. However Smyly (1937) states it has occasionally been found to contain *B. dysenteriae* and Elworthy has recorded that on one occasion he isolated *B. dysenteriae* from the synovial fluid. He employed a large amount of joint exudate but only 4 colonies of the organism appeared on the plates. Klein and Zia and Smyly (1931) have noted that inflammatory fluid from the joint may have the property of agglutinating the dysentery bacillus in a titer even higher than that given by the blood serum.

The joint swelling usually eventually clears up completely and apparently the fluid has never been reported purulent in character nor have heart lesions been observed in connection with dysentery arthritis. Some of the reported joint involvements have in great probability resulted from serum reactions from the anti dysentery serum used for treatment.

Clifford has reported 7 cases of arthritis deformans in 4 of which there was a history of a dysentery due to Flexner type bacilli. He suggests that mild cases which have not

been recognized as dysentery may have been caused by dysentery bacilli and suggests agglutination studies in arthritis cases where no distinct history of dysentery is obtainable. Cope in Salonica has also noted an ankylosing type of arthritis with periarticular thickening resembling true rheumatoid arthritis. In addition to the arthritis there may be neuritis which in severe cases may go on to muscular atrophy.

Parotitis due to secondary infection perhaps through the mouth and throat has been frequently noted as a complication. Rarely conjunctivitis and iridocyclitis may occur. Subnormal temperature may follow severe attacks.

In some epidemics of dysentery *gangrenous manifestations* have been common. This is a very fatal type that is recognized by the passage of dark brown serous discharges containing ashy grey to black sloughs or even tubules of gangrenous mucosa, the stool having a putrid odor. The general symptoms are pronounced, there being a dry glazed tongue and low muttering delirium with a thready pulse. The condition resembles the typhoid state.

**Chronic Cases**—It has been usual to consider bacillary dysentery generally as a self limited disease running on to convalescence within 10 days to 2 weeks. Rogers has called attention to the importance of bearing in mind a chronic condition especially in natives as well as an acute one. In these chronic cases the ulcerations are usually located in the descending colon sigmoid flexure or rectum and give rise to frequent stools containing blood and mucus and causing a progressive loss of strength and weight. There is marked digestive disorder and the patient becomes weak anaemic and neurasthenic. In such cases stenosis of the large intestine may result and atony of the large bowel with post dysentery constipation. Manson Bahr and others have observed a post dysentery form of dyspepsia in which achlorhydria and hypochlorhydria have been found.

Lobar or bronchial pneumonia is a not infrequent terminal event in the chronic form of dysentery.

### SONNE DYSENTERY

The Sonne bacillus was first reported by Kruse and his associates who described it as a lactose fermenting or E race of pseudo-dysentery bacilli. In 1904 a lactose fermenting organism was found by Duval in the United States and in 1915 Sonne gave a detailed description of it during an outbreak of dysentery in Copenhagen. Subsequently it was found especially in England, Egypt, Australia, Brazil and since 1930 in a number of outbreaks in the United States. Several small epidemics in New York State and elsewhere have been reported by Gilbert and Coleman (1929) and Leahy (1931). In Japan it has been said to have caused acute dysentery in children which is known under the name of *ekiri*.

The organism is non motile and resembles in morphological characteristics the dysentery bacilli. On agar 2 forms of colonies have been observed one round and smooth and the other flat and irregular. The different colonies may vary in agglutination; smooth colonies giving different reactions from rough ones. On lactose litmus agar the colonies are at first bluish and later reddish. The litmus milk remains unchanged at first but lactic acid is produced and it gives a negative methyl red reaction. On McConkey's medium the colonies frequently show a small central point of acidity on the somewhat opaque background. Mutations of the organism have been

observed and great variability has been described in the serological properties of different strains and at times in some of the reactions in sugars. The primary cultures are not agglutinated by specific serum after 4 hours at 55 C. however subcultures later may become agglutinable and all strains absorb agglutinins. It has not been demonstrated that serum prepared from Flexner's strains contain agglutinins for the Sonne bacillus.

Reynolds (1924) has studied the dissimilation of lactose and sucrose by the Sonne type of organism. He found both alkaline and acid colonies suggesting changes in the metabolism of the organism accompanied by changes in the production of carbon dioxide, acetic acid and ethyl alcohol. The organism has very frequently been isolated in cases of intestinal disturbance. Whether it alone is of important etiological significance in dysentery has not been conclusively demonstrated. It has been suggested that the organism may have produced toxins in the food ingested.

**Clinical Observations**—Descriptions of the clinical manifestations reported especially in England and in the United States during recent years, in which the Sonne Duval bacillus has been found have varied greatly. Kinloch and Smith (1906) reported on one type in which the symptoms approximated those of acute dysentery with sudden onset diarrhoea, colic and the appearance of blood and mucus in the stools. In the second type the cases assumed a more alarming aspect and approached in virulence cases of infection with the *Salmonella* type. Charles and Warren (1929) described cases with sudden onset vomiting and diarrhoea with stools more nearly resembling the choleraic form of Shiga dysentery followed by rapid prostration. Manson Bahr (1939) reports that in the great majority of Sonne infections the symptoms appear suddenly resembling an irregular diarrhoea with greenish mucoid stools. There is a tendency to fever but in the milder cases the fever is slight. Catarrh of the respiratory system has been frequently observed in association with diarrhoea. The stools usually number 5 to 8 in 24 hours, the acute symptoms generally persisting for 48 hours. The stools remain loose and greenish, but in the course of a few days generally become brown and formed. Harvey (1933) has found that the acute infection in children under 9 years of age may be a cause of sudden death. Hay (1930) has reported 2 fatal cases in the United States.

A considerable number of outbreaks of food poisoning have been reported in Great Britain during the past few years in which the Sonne bacillus has been isolated. Whether some additional inciting factor was sometimes present is not clear. Snowden (1933) reported an outbreak among 13 people who had eaten a pea-se pudding. Five were adults and 8 children. Two of the children died. He stated that Sonne's bacillus was isolated from some of the pudding and it was suggested that the acute symptoms produced were due rather to the toxins of the organism which had germinated in the pudding. A number of outbreaks have been reported in institutions and Fyfe and Bowes (1938) have reported outbreaks which were definitely milk-borne. In Fyfe's outbreak there were 200 cases. The incubation period was probably less than 12 hours. Recovery was usually complete in a week. In the outbreak of dysentery reported by Bowes the organism was isolated from the milk as well as from the faeces of the patient. Fifty-nine of 106 households were affected.

In the United States Nelson has found this infection to be wide spread in children in Boston. McGinnis and his associates (1936) in the study of 300 cases of diarrhoea and dysentery in Virginia found 50 of the cases due to the Duval Sonne and 100 due to Flexner's strains.

Silverman (1937) has suggested a new method for the diagnosis of chronic cases of dysentery of lactos fermenting organisms by feeding milk containing the acidophilus bacillus. He reports that this changes the reaction of the content of the large bowel. Sonne bacilli appeared in the cultures usually after the third week of such feeding which were not found in the cultures before.

### DIAGNOSIS

In the presence of the dysenteric syndrome of tormina, tenesmus, frequent scanty stools of muco purulent or muco sanguinolent character, one must keep in mind the various conditions which may give rise to such manifestations of dysentery and not make a diagnosis of bacillary dysentery until we have excluded amoebic infection, tuberculous, cancerous and syphilitic processes, as well as those connected with schistosome or other helminthic infections. It is well to keep in mind that amoebic and bacillary infections may be associated in the same case.

**Clinical Diagnosis**—The clinical diagnosis of bacillary dysentery except in some cases of the acute fulminating type is often difficult. While bacillary dysentery usually occurs as an independent infection, it is not infrequently found as a terminal infection in a number of chronic wasting diseases such as pellagra, scurvy and phthisis, as well as in the parasitic infectious diseases kala azar, schistosomiasis and amoebic dysentery. Hence care must be taken to confirm or exclude the presence of such diseases. In pyrexial cases the possibility of infection with the paratyphoid organisms and *Salmonella entericus* infection must be kept in mind. Malaria also may be present as a complicating disease and dysentery may cause a latent malarial infection to develop.

Amoebic dysentery may sometimes be differentiated clinically from bacillary dysentery by the usual absence of manifestations of toxæmia and by its insidious onset and chronic course. It is important, however, to remember that either bacillary or amoebic dysentery may show gangrenous manifestations and in such cases the clinical picture may be very much the same whether the process is amoebic or bacillary. Fulminant bacillary dysentery may greatly resemble cholera in its algid stage although there is absence of typical rice water stools in dysentery and usually blood is present. The simple microscopical examination of the dejecta may suggest strongly the diagnosis particularly if no motile amoebae are present. However, a definite diagnosis can only be made in some instances by isolating the dysentery bacillus in the acute stages of the disease from the intestine or stool by plate cultures.

Typical liver abscess is a complication exclusively occurring in the amoebic form of dysentery while joint manifestations and evidences of multiple neuritis may be noted in some epidemics of bacillary dysentery. Again the toxins of the dysentery bacilli have a tendency to damage the myocardium. The good effects of the administration of emetine is in favor of the diagnosis of amoebic dysentery.

There is usually an absence of fecal material in the stools in acute bacillary dysentery and the bulk of the stool is composed of clear or turbid amber-colored serous fluid which float curled masses of white mucus flecked with bright red blood. While in amoebic dysentery the typical stool contains faecal material and mucus and blood. It has been shown also that the microscopic appearance of the stools of these 2 types

of dysentery differ. In fact a provisional diagnosis of bacillary dysentery can often be made by the microscopical examination of the stools or from material obtained from the rectum. In the case of the stools 2 requisites are important: the specimen must be freshly passed and early in the course of the disease. After several hours the material usually becomes valueless for examination. Callender (1944) emphasizes that in diagnosis the stool should be examined (1) for the presence of exudate (2) for the character of the exudate (3) for the presence of protozoan or other parasites and (4) bacteriologically for the causative organism. The doing of any one of this group without the other leads to a false picture of the condition present and has made our statistical data essentially worthless.

**Laboratory Diagnosis**—Usually the chief point in diagnosis is to determine whether we are dealing with amoebic or bacillary infection. While these two kinds of dysentery rarely may coexist it is logical for the clinician to consider a case in which there are found amoebae with long rapidly extruded finger-like pseudopodia and containing red blood cells as one of amoebic dysentery.

A fresh specimen of the muco-purulent stool of bacillary dysentery shows in addition to pus cells numerous large endothelial macrophages. Some of these may show vacuolation and in some instances even red blood corpuscles and may resemble considerably amoebae. Occasionally leucocytes may be observed in their cytoplasm as well as granules and fat globules. The macrophages may measure 10 to 45  $\mu$  in diameter and may be round, oval or bilobed. Such cells never show motility but under conditions of lowered temperature of the specimen or from prolonged standing and beginning disintegration the amoebae too may fail to show motility. Warm stage preparations should be searched for amoebae.

**Differentiating Stain**—If specimens are mounted in Gram's iodine solution these large macrophages show a much larger nucleus than that which occurs in amoebae and take the yellow staining of iodine more intensely. The most suitable procedure however is to make a smear, fix it lightly by heat and stain by Gram's method or with Loeffler's methylene blue or dilute carbol fuchsin. These large phagocytic cells stain easily and well and in the Gram specimen there sometimes may be observed Gram-negative bacilli in the cytoplasm. Giemsa's stain with methyl alcohol fixation or the usual Wright or Leishman technique answer equally well. On the other hand it is rather difficult to obtain satisfactorily stained amoebae in this way, it usually being necessary to fix moist thin smears of the stool with some bichloride fixative as Schaudinn's fluid and then carry out the staining with haematoxylin.

The presence of pus cells as well as endothelial cells in a stained smear of material from a bacillary dysentery stool is of value in differentiating from an amoebic stool smear in which pus cells are rarely seen. The amoebic dysentery smear gives more the picture of granular debris. More than 95 per cent of the cells of the smear from bacillary dysentery are polymorphonuclears which show signs of degeneration as indicated by swelling and a ring type of nucleus due to accumulation of chromatin at the periphery (ghost cells). However at other times the smear may show these cells unchanged and not unlike those seen in a fresh pus smear. The red cells more generally are not clumped while in an amoebic dysentery smear we often note small clumps of red cells. Columnar epithelium cells, mononuclear leucocytes and lymphocytes may commonly be observed in the specimens. It should be emphasized that one should make these smears and examinations and also prepare cultures at the onset of the dysentery as these differentiating characteristics and the dysentery bacilli themselves may disappear later on in the disease and one should always examine a stool as soon after it is passed as possible.

**Cultivation**—If the microscopical examination suggests a bacillary infection we should take a small portion of the stool containing mucus, wash it in sterile water and then drop it in a tube of sterile bouillon or salt solution. After emulsifying in this tube of bouillon one may take up 2 or 3 loopfuls of the suspension and deposit them on the surface of a litmus lactose agar plate, later spreading out with a glass rod either by successive parallel strokes or by revolving the plate while smearing the surface with the glass rod. Other cultures should be made by spreading on the surface in parallel lines.

over several plates a little mucus directly from the stool. It is in the first 2 or 3 days of an attack of acute dysentery that we obtain the best cultural results occasionally obtaining a very large number of colonies of dysentery bacilli from proper material taken at the onset. The writer has never obtained a pure culture of *Bacillus dysenteriae* from the stool or intestine even in the most acute cases. Manson Bahr stated that he had never recovered true dysentery bacilli from a purely faecal stool. Even faecal contamination of the mucoid mass makes it difficult to recover the organism. Dysentery bacilli rapidly die out if the stool is acid so that it has been recommended to make the stool alkaline (with an equal volume of N/33 NaOH solution) where it has to be sent to a laboratory from a distance.

In making plate cultures L-timus lactose agar sometimes gives better results than the more restraining faeces plating media. However Endo's fuchsin agar may be employed because it is usually at hand for typhoid or paratyphoid examinations in many laboratories. The dysentery bacillus colonies on this medium are like those of typhoid—greyish white. In England some observers prefer MacConkey's neutral red bile salt agar while others use the Conrad Drigalski medium. The Teague medium is also very satisfactory.

Other media especially recommended are the Deso's chocolate Citrate Agar the Bacto Bismuth Sulphite Agar and the Bacto S-S Agar. The last is prepared by the Difco Laboratories and is a selective medium designed to provide differentiation of lactose fermenting from lactose non fermenting organisms and to give a maximum inhibition of clostridial organisms with a minimum restriction of growth of the pathogenic gram negative intestinal bacilli from specimens of the faeces. However since it is a selective medium a non selective one such as MacConkey's should be employed at the same time in order to be sure that all gram negative intestinal pathogenic organisms are restricted. Shiga stains sometimes grow very slowly on the S-S agar and appear as minute pinpoint colonies. Wells and Blair (1941) have found a tellurite iron resorcinol acid medium as especially selective for Flexner strains of *B. dysenteriae*.

On L-timus lactose agar the dysentery bacillus colonies are like those of typhoid greyish white or bluish grey dew-drop like in appearance and about 1 mm in diameter. The colour of the agar about them does not change as it does with the colonies of colon bacilli scattered about the plates. About some of the Hiss Russell bacillus colonies there is sometimes a pale reddish violet tint. The Sonne bacillus colonies are at first bluish and later of a red tint.

On all these media the colonies resemble more or less those of typhoid and the differentiation is aided by examining for motility. At the same time one not infrequently finds lack of motility in bacilli from colonies just isolated on Endo's medium which later on in subculture show motility and are found to belong to the typhoid or paratyphoid group. For the accurate determination of dysentery bacilli or for differentiating the Flexner Shiga and Sonne strains one should carry out agglutination tests with different immune sera. For practical diagnosis a polyvalent immune serum may be employed that has been prepared with at least four of the common mannite fermenting strains and a Shiga nonfermenting one. If any organism isolated on the plate inoculated from the faeces agglutinates with such a serum in dilutions of 1:50 or 1:100 the case may be regarded as one of bacillary dysentery.

The isolation of dysentery bacilli from chronic cases or from convalescents as intimated is more difficult as a rule and agglutination tests with the patients' serum may be of more value in some cases. In chronic cases the exposure of the ulcer where possible as by the proctoscope or sigmoidoscope and the plating of scrapings from its base may give the best chance of recovering the organism. During the World War the examination of material obtained by rectal swabs was extensively employed and results were said to be more satisfactory than those obtained from culturing the stools. Smyly (1937) reports that he has obtained cultures from swabs directly from the ulcers in a large proportion of such cases. Ziem's glass tube (3 cm long by 2 cm in diameter with a lateral opening 3 cm above the lower closed end) for obtaining material from the sigmoid flexure has also been recommended.

**Agglutination Test.**—Manson Bahr (1939) who has had wide experience with this reaction points out that it is a matter of disappointment



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the rule for a para dysentery type bacillus to show greater specificity for its own serum and the Shiga type greater specificity for a serum prepared with the more toxic non acid strains. Thomas Mackie and his associates (1938) in the diagnostic study of cases in N. Y. City have emphasized that prolonged serologic and cultural studies indicate the necessity for cautious interpretation of the agglutination reaction in cases of chronic inflammatory disease of the colon. They have repeatedly found agglutinins for *S. dysenteriae* present at titers commonly considered to establish the diagnosis unaccompanied by cultural evidence of homologous infection. Conversely no agglutination reactions have been observed in cases of proved infection. Marked and unaccountable variations of agglutinin titer were the rule in the course of repeated determinations. It is a striking fact that the majority of the serums which gave a high titer were obtained from patients who consistently showed sterile culture for *S. dysenteriae*. These observations suggested that agglutinins for *S. dysenteriae* may develop in response to non specific heterologous stimuli.

In a later paper Mackie also pointed out that a close agglutinogenic relationship may exist between certain strains of *E. coli* and *Shigella dysenteriae* as Sonne and Flexner strains. He has found that the agglutination reaction is unsupported by confirmatory bacteriological evidence so it constitutes valid proof of infection by these organisms and that the diagnosis of bacillary dysentery can be substantiated only by the demonstration of the organism itself.

In performing the agglutination test for diagnosis with the patient's serum it is recommended that one employ at least 4 of the common mannite fermenting strains and a Shiga non fermenting strain as results obtained with one may differ from that obtained with another. Agglutination absorption tests are usually necessary to determine the precise type. The statement of Willmore and Savage and concurred in recently by other observers that the differential action of the different types of dysentery bacilli is a refinement of technique for interested bacteriologists seems a proper view because if a polyvalent serum is employed for treatment one only needs to know that the case is one of bacillary dysentery. Of course with a monovalent serum prepared only with the Shiga bacillus one would have to determine whether the organism producing the dysentery was of that strain. However it must be borne in mind that a Shiga immune serum will also agglutinate Flexner and Strong strains.

The identification of the infecting strain may be of some possible interest in prognosis. A Shiga strain is frequently but not invariably much more toxic than the acid strains. However the clinician should realize that as a matter of fact it frequently takes considerable time and laboratory skill to carry out reliable cultural and serological tests. From a practical standpoint for diagnosis one can use a polyvalent immune serum prepared for therapeutic use for making agglutination tests and if any organism recovered on the plate culture made from the faeces agglutinates in 1 to 50 or 100 this may be considered suggestive of bacillary dysentery rather than of amoebic infection.

Often one does not see a case of dysentery until late in the disease and then provided the condition is serious and the diagnosis points to a bacillary infection if one decides to employ serum treatment it would be better to inject it at once rather than await laboratory confirmation.

**Bacteriophage**—Durrell Feemster (1934) Winkelstein (1935) Feisen (1936) and Millis (1937) have employed bacteriophage as an aid to diagnosis. Millis has emphasized its importance for a quick diagnosis by its action on different types of dysentery bacilli. Feemster in the study of an institutional outbreak examined bacteriologically 100 cases in which the Hiss Y dysentery bacillus from cultural tests appeared to have been the chief etiologic factor. This organism however was isolated from only 5.6 per cent of the stools collected from the sick patients. On the other hand bacteriophage of the Hiss Y dysentery bacillus was found in 80 per cent of the stools taken during the second week after the onset and in 45 per cent of those collected during the third week. The longest time after the onset that phage was demonstrated was 63 days. None was found in the stools of the control group which had not been ill nor in specimens obtained 6 months after the attack from patients who had

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that serological diagnosis which is usually so satisfactory with the typhoid group is such a comparatively unstable weapon in dysentery. The writer in the study of the test especially in the Philippine Islands (1900-1912) also found the agglutination test frequently unsatisfactory for the diagnosis of bacillary dysentery. It is generally accepted that the macroscope method in the test tube should be employed, the tubes being incubated for 2 hours at 55°C.

Although some observers have reported the appearance of agglutinins in the serum of cases of acute bacillary dysentery within 3 or 4 days from the onset of the disease, yet it is unusual to obtain agglutination with the patient's serum before the 10th day.

The agglutinins generally appear about the 7th and may reach their maximum about the 21st day after which there may be a rapid decline. In other cases however some residual agglutinin may persist in the serum for a considerable time and it has been claimed that they could be demonstrated after as long an interval as 3½ years. Dudgeon (1918) in studying this reaction in Salonica during the World War found the highest agglutinin titers during the second and third weeks of the disease. The limitations of the test are therefore obvious and in the most acute cases where an early diagnosis is important it is generally of no value. With the Shiga strains an agglutinating power in 1 to 50 has been usually accepted as evidence of specificity but for Flexner strains a higher titer is regarded as necessary for diagnosis and a dilution of 1 to 150 should be required for the test.

Ritchie tested the sera of 792 normal persons and found that 30 per cent of these individuals agglutinated Shiga bacilli in 1 to 32 while with Flexner strains 41 per cent agglutinated in 1 to 64 and 30 per cent in 1 to 128. For comparison Ritchie's results with typhoid showed that only 6 per cent agglutinated such bacilli in 1 to 16. There is some evidence that typhoid vaccination increases the agglutinating power of the serum against dysentery organisms. The usual advice is to consider an agglutination of 1 to 40 as fairly specific for Shiga infections and 1 to 100 for paradysentery ones. Gardner (1923) also showed that some normal sera will agglutinate the organisms of the Flexner group so that normal serum should always also be tested with the dysentery organism as a control.

Browne (1937) in New Orleans during a study of agglutination reactions found frequently that the blood of normal individuals agglutinates stock cultures of *Bacillus dysenteriae* such as the metadysenteriae bacilli of Castellani and the Duval lactose fermenter. In certain instances the normal blood titer ran as high as 1:300. He therefore believes that the positive agglutination of the stock culture of *Bacillus dysenteriae* of dilutions of 1:160 is not in all instances sufficient evidence to warrant a diagnosis of bacillary dysentery. He was able to isolate one or more of the dysentery organisms in 8 per cent of normal individuals, the most frequent one being the lactose fermenter of Duval.

Speares and Delono (1919) found that the serological diagnosis was not always possible in mild infections and that the injection of immune dysentery serum had no appreciable effect on the agglutination titer of the patient's serum.

Willmore and Savage tried heating serum to 55°C for 30 minutes but found that such a procedure was of no practical value with dysentery thus differing from Malta fever serum where such a procedure is of value in destroying coagglutinins and thus increasing the specific action. The work of Ohno would indicate that we should trust to the acid producing effect on mannite for differentiating Flexner and Shiga strains rather than on agglutination because it was found that agglutinins for an acid strain were not always more specific for such strains than for non acid ones. However more recent work has shown that also the sugar reactions may be inconstant with the strain and may change after prolonged cultivation. Nevertheless group agglutination among the different serological races is a conspicuous phenomena. At the same time it is

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**Bacteriophage**—Durrell Feemster (1934) Winkelstein (1935) Felsen (1936) and Millis (1937) have employed bacteriophage as an aid to diagnosis. Millis has emphasized its importance for a quick diagnosis by its action on different types of dysentery bacilli. Feemster in the study of an institutional outbreak examined bacteriologically 100 cases in which the Hiss X dysentery bacillus from cultural tests appeared to have been the chief etiologic factor. This organism however was isolated from only 5.6 per cent of the stools collected from the sick patients. On the other hand bacteriophage of the Hiss X dysentery bacillus was found in 80 per cent of the stool taken during the second week after the onset and in 45 per cent of those collected during the third week. The longest time after the onset that phage was demonstrated was 63 days. None was found in the stools of the control group which had not been ill nor in specimens obtained 6 months after the attack from patients who had recovered. Feemster concluded that the detection of the bacteriophage

action against the bacillus causing outbreaks of dysentery seems to be a supplemental and valuable procedure for determining their etiology

Winkelstein (1937) and Felsen have also obtained a diagnostic bacteriophage from certain cases of chronic ulcerative colitis. Winkelstein and Herschberger found a phage present in 36 per cent of 41 cases of ulcerative colitis and absent in 45 miscellaneous controls. They thought that its presence gave indirect but suggestive evidence that the patients have had or are suffering from bacillary dysentery. Mackie (1936) studied the diagnostic significance of antidysentery bacteriophage in 170 individuals with chronic ulcerative colitis. Antidysentery bacteriophage was found in 29.1 per cent or 55 cases presenting acceptable evidence of chronic infection by *Bacillus dysenteriae*. However an antidysentery bacteriophage was found in 12.8 per cent or 86 miscellaneous cases which presented no evidence of infection with *B. dysenteriae*. He concluded that in chronic intestinal infection the term diagnostic bacteriophage was not justified. Gupta (1930) moreover believes that a phage active against *B. dysenteriae* frequently can be isolated from individuals not suffering from dysentery. There seems to be no further progress in the practical use of bacteriophage for diagnosis.

Intradermal reactions in bacillary dysentery have been studied by Brokman (1923) and Soeller (1927) the reaction being applied as in the Shuck test 0.1 to 0.2 cc of a 1:100 dilution of dysentery toxin being injected.

Brokman observed reaction beginning after 24 hours while Zoeller reported that the specific reaction did not appear until the third or fourth day when an ecchymotic tinge became pronounced and led to the formation of a small black slough. A negative reaction was said to indicate the presence of sufficient antitoxin to neutralize the toxin. These tests have not been demonstrated to be of value in the study of bacillary dysentery.

**Diagnosis with the Sigmoidoscope**—Biggam (1930) Paulson (1930) Manson Bahr (1936) and Smyly (1937) have particularly studied the sigmoidoscopic diagnosis. Manson Bahr points out that it is not often necessary to use it in making a diagnosis of bacillary dysentery in the early and acute stages and that it is doubtful whether its use is always justifiable on account of the pain of introducing the instrument in the acute cases and since the damage that results may be considerable. Also it cannot be used without employing a general anaesthetic. In the later stages of the disease a cathartic of ½ oz. castor oil should be given the night before and the following morning before the instrument is introduced the bowel is cleared out with a warm water enema. Ten to 16 m. of tincture of opium is generally given half an hour before the examination.

In many of the chronic cases no suitable ulcerations can be discovered in the rectum or those parts of the colon which can be seen by the instrument. The examination in many cases shows that the mucosa is profusely red and either finely or coarsely granular. When coagulation necrosis has developed it may have a greyish green necrotic appearance with haemorrhagic areas. Actual ulceration may be often observed the commonest type being a very shallow ulcer the margin of which is sharply defined and ranging in size from 1 millimeter or less to over a centimeter. The base is usually covered with pus which is easily swabbed away leaving a surface of red granular tissue. Microscopical preparations and cultures may be made directly from such ulcer. In the chronic cases a rough granular mucosa is often seen.

**X-ray Diagnosis**—In the acute stage of bacillary dysentery barium enemata and X-ray diagnosis are of practically no value. In the chronic disease the appearance is sometimes suggestive of a sub acute or chronic ulcerative colitis in that certain filling defects may be seen in the colon. However generally little of value is obtained from the X-ray examination.

### PROGNOSIS

The mortality varies greatly in different epidemics. The prognosis may depend upon the severity of the epidemic the age and general con-

dition of the patient and the presence or absence of complications. Prognosis is bad when the intestinal symptoms are very severe and persistent and collapse has resulted. Cases showing greenish sloughs of mucosa are most serious. Persistent hiccough and vomiting are most unfavorable signs. Any complicating disease—malaria, nephritis or pulmonary infection—renders the outlook less favorable. The prognosis is also unfavorable in severe cases in white children in the tropics. In some of the epidemics in Japan the mortality has been high, reaching 50 per cent but averaging about 25 per cent. During inter epidemic years it has been considerably lower. In one epidemic in debilitated natives of the Solomon Islands the mortality was given as 47 per cent. In well nourished and otherwise healthy individuals the mortality is lower. Thus during the World War in British forces in Macedonia, Egypt and Mesopotamia it did not exceed 2.7 per cent. In many of these cases the reported symptoms were mild, the diarrhoea lasting not more than 8 days. Manson Bahr (1936) states that of the many thousands of cases in the British troops it is doubtful if the case mortality at any period rose above 5 per cent, which is evidence of an epidemic of not great virulence. In the German Army during the World War 20 per cent of the deaths were from dysentery. In a series of 525 cases in Germany during 1916–20 reported by Froemsdorff (1923) 415 recovered or 79.04 per cent, 22 were improved 4.19 per cent and 86 died 16.38 per cent. The death rate was higher in elderly patients and in those debilitated as a result of war privations. In the epidemic in Mecklenberg in 1938 the mortality rate was 12 per cent. In a series of 200 private cases quoted by Manson Bahr where the subsequent course could be traced over a period of 6 years 3 or 1.43 per cent became chronic.

#### TREATMENT

In the treatment of bacillary dysentery absolute rest in bed is important to keep up the strength of the patient and also to protect the heart, which tends to be more or less damaged by the toxic action of the dysentery bacillus. Some prefer to prop up the patient in bed, considering a strict dorsal decubitus as undesirable. Such a position may lessen the amount of air swallowed in frequent administration of nourishment. It is important to use sufficient covering on the patient to avoid chilling. A light wool blanket spread over the abdomen is often all that is needed in the tropics.

The patient should not be allowed to get out of bed to defecate and in severe cases should not be allowed to exhaust his strength by straining on a bed pan. A waterproof sheet should be used, which can be changed and cleansed frequently and the buttocks may be packed with carbolized cotton wool or tow, which should be changed frequently and burned. In very severe cases there may be incontinence of both urine and faeces. Nurses and other attendants should wear rubber gloves for protection.

**Diet.**—Solid food is not permissible. In the acute dysentery stages the diet should consist of albumin water or barley water sweetened with lactose. This is to be preferred to milk, which is usually not well borne.

by many patients. Kendall has especially noted the value of lactose in lessening the toxicity of various organisms. Tea sweetened with lactose is usually well borne. The liquid diet should be warm and given only in small quantities and frequently as it may cause increased peristalsis and increase the number of evacuations. When the most acute symptoms have subsided, meat juice expressed from a piece of lightly broiled steak is of value. Various jellies or sago pudding may also be given. Most authorities agree that milk is badly borne and that after it solid curds of casein are often passed in the stools. However, citrated milk is often well tolerated. Korner (1935) recommends ice cream in place of milk. Vitamins such as ascorbic acid or thiamin hydrochloride may be administered during convalescence if there is evidence of such vitamin deficiency.

When mucus and blood have disappeared entirely, the diet may be gradually increased by adding lightly poached eggs, custards, milk, pudding, toast and butter. Especially during the convalescence care must be exercised and the return to normal diet, with fish, chicken and vegetables, should be very gradual.

**Medical Treatment**—Most authorities recommend a preliminary dose of  $\frac{1}{2}$  oz (15 cc) of castor oil to which may be added 15 minims (1 cc) of tincture opii. This is given in order to clear the large intestine of any remaining faecal contents. In milder cases when seen early some have advised about 2 gr. of calomel in divided doses of  $\frac{1}{4}$  gr. every half hour then followed up with saline treatment. Others recommend treatment with castor oil or rhubarb which is given in dram doses hourly for 8 doses until the third or fourth day of illness with the idea of keeping up a constant peristaltic action. Subsequently salines are given.

Saline treatment has been widely recommended by many authorities. Sodium sulphate in saturated solution may be given 1 to 2 drams (4-8 cc) every 1 or 2 hours when the patient is awake for the first 24 hours and afterward every 4 hours until the stools become faeculent. Some physicians are opposed to purgative treatment especially on the ground that it increases peristalsis and hence favors the extension of the intestinal lesions and the action of all bacteria which may be present in the intestines.

Talbot (1937) in the treatment of infants says that at the outset the bowel should be cleared with castor oil or 1 grain of calomel in divided doses given if there is any distention or evidence of retention of toxic faecal material. If on the other hand the abdomen is flat or sunken and there is no evidence of faecal retention cathartics should not be given.

Korner (1935) believes that an initial purge is not advisable for patients who have had severe diarrhoea before being seen by the physician and in severe cases of incontinence of faeces its use is questionable. Smyly (1937) cautions that in children or adults suffering from dehydration sulphates must be given cautiously abundant fluid by mouth is advised and also when necessary by intravenous or subcutaneous injection. In pregnant women there is danger of abortion in bacillary dysentery and the use of aperient sulphates must be carefully watched.

Manson Bahr (1939) has found that some cases of the disease do not tolerate saline aperients well and in these calomel  $\frac{1}{2}$  grain every hour for 12 hours on 3 consecutive days may be used. Others Welch, Mascarenhas and Boase believe that castor oil treatment is more efficient than the use of salines. 2 oz. was given on the first day and 1 dram hourly during the day time on the second and third day. They consider the small doses keep up gentle and continuous peristaltic action and that the contents of the intestine is thus passed on and that the toxic action is thereby reduced.

**Morphine**—Especially in severe fulminating cases it is frequently necessary both for the relief of pain and to forestall collapse to attempt to limit the peristalsis and give rest to the inflamed colon by hypodermic injections of  $\frac{1}{4}$  g. (0.016 gm.) of morphine sulphate every 3 or 4 hours. Undoubtedly many acute cases have been saved by its use. Some observers have suggested for theoretical reasons that it may be liable to increase toxæmia. However the danger of death from collapse due to physical exhaustion from excessive straining and the nervous shock from increased peristalsis and constant bowel movements in many cases is much greater than the danger of the exacerbation of toxic symptoms. Theoretically also if the amount of toxine is slowly absorbed recovery from the disease may be favorably influenced by the production of antitoxin in the body.

**Sulfamidyl guanadine**—This new synthetic preparation is especially recommended for treatment of bacillary dysentery on account of the destructive action it exerts upon the dysentery bacilli in the intestinal tract. Particularly from experimental observations on animals Marshall believes that with a dosage that can be safely employed in man a low concentration of the drug in the blood may be obtained. He and his associates (1941) have reported upon 17 cases of acute bacillary dysentery in children treated at the Johns Hopkins Hospital with sulfamidyl guanadine. In 10 of this group of 17 children the stool cultures became negative for dysentery organisms during treatment and remained negative during hospitalization. In 5 other cases the stools became negative on the last day of therapy or in two days after its discontinuance. Marshall recommends the further trial of this drug in acute bacillary dysentery in children. An initial dose per os of 0.10 gram per kilogram is given and a maintenance dose 0.05 gram per kilogram every 4 hours until the number of stools per day is 4 or less then 0.10 grams per kilogram every 8 hours for at least 3 days. The finely powdered drug is administered in milk or in water suspension. It is imperative that the drug should not be continued longer than 14 days as it seems doubtful if longer treatment will be beneficial. Furthermore he points out that this limitation of treatment minimizes the possibility of agranulocytosis which with other sulfamidamide drugs does not occur before the 14th day.

The dose schedule in adults recommended is the same as for children except that when the drug is given every 8 hours 0.05 gram per kilogram is given instead of 0.10 gram per kilogram.

No definite toxic effects were observed in the series of 23 children given the drug although occasionally vomiting was present for a short period after chemotherapy. As soon as the general condition improved the vomiting ceased although the drug was continued. None of the



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*Sulfadiazine*.—Now, however (1944) based especially upon the studies of Hardy in the treatment of mild cases of bacillary dysentery occurring in New York State institutions, *sulfadiazine* is regarded as the drug of choice for initial treatment. The dosage is 1 gram four times daily. Two days after clinical recovery, sulfonamide therapy should be stopped. If after two additional days there has been no recurrence the patient may be discharged. The fluid intake should be 3,000 cc and sufficient fluids must be administered to insure a daily urinary output of at least 1,500 cc.

Ravdin and Norfleet (1943) and Nagel (1943) and Coghill (1943) are among those who have emphasized the danger of the use of sulfadiazine in the tropics where large amounts of fluids are lost through the skin. Gross and microscopic crystals have repeatedly been found in patients with urinary outputs of from 1,000 to 1,200 cc. Renal complications may also occur in temperate climates.

Lapping (1942) has found sulphapyridine very satisfactory in the treatment of 56 cases in India. Treatment was commenced with 2 gm and was continued with 1 gm 3 hourly until general symptoms subsided. The drug relieved pain within 12 to 18 hours. The stools became normal within 3 to 7 days. The earlier in the disease the drug was given the quicker the response there was. There was only one death in a marasmic child 2 years of age suffering also from Malaria.

Doriman and his associates (1940) found there is an inhibition of respiration of dysentery bacilli by sulfapyridine. Their results indicate that the action of sulfapyridine on micro organisms may be related to the role of nicotinamide in their metabolism.

*Collapse*.—In severe cases the pulse and blood pressure must be watched and should there be evidence of approaching collapse as indicated by a sudden fall in the pressure or from the character of the pulse attention must be given to keeping the patient warm. Brandy may be given by the mouth and intravenous injections of saline solution employed. Favorable results have been reported from 5 per cent glucose in 500 cc normal saline solution allowed to run in slowly the injection to take not less than a half hour. Others have preferred injections of normal saline solution in larger amounts from 1,000 to 2,000 cc being injected. In cases with marked dehydration good results have been reported from the use of Rogers hypertonic cholera solution (see under cholera). All solutions for intravenous injection must be very carefully sterilized and introduced slowly at the rate of not more than 4 oz (115 cc) a minute at a temperature of 104 F (40 C).

children developed rashes or had drug fever. No haematuria was seen except in one case where it was very transitory.

Marshall believes that saturation of the intestinal contents with the drug can be obtained with the dosage which gives a low concentration in the blood thus confirming deductions made from experimental observations on animals.

Lyon (1941) has treated with this drug 20 cases of severe acute bacillary dysentery in West Virginia and 20 cases of a similar severity and clinical picture were observed as untreated controls. Alternate cases were treated. In the treated cases 5 seemed to follow the general course of the untreated cases. One case showed a good therapeutic response and 14 showed excellent therapeutic response. Many recoveries were most dramatic in character. The cases generally showed a rather rapid fall in temperature and leucocyte count within 48-72 hours and a closely paralleling general clinical improvement and a marked reduction in the number of the diarrhoeal stools and improvement in their character in the first 48-72 hours after institution of the chemotherapy.

A large supply of this drug has been very recently sent from the United States to Cairo for the use of the British armies in the Near East and Africa.

Colonel Hamilton Fairley and Colonel J. S. K. Boyd (1942) have reported upon the treatment of 371 cases in the British and Australian troops in the Near East. They found that cases of bacillary dysentery due to *Shiga Flexner Schmitz Sonne* strains treated with sulfaguanidine have been restored to health and rapid healing of the various types of colonic lesions has been observed by the sigmoidoscope. The only cases in which death occurred had either received treatment too late or had suffered from some complication such as pneumonia or peritonitis and toxic nephritis established before treatment was started. Many other reports have been published indicating favorable results in the treatment of the disease with sulfaguanidine in the United States, North Africa and the Southwest Pacific.

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Poth and Knotts have suggested that Succinyl Sulfathiazole may be a superior drug to sulfaguanidine for the treatment of dysentery. However, this drug has not yet been sufficiently tried to demonstrate whether or not it is as efficacious or superior to sulfaguanidine.

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tions and they are liable to cause argyria. For this reason if an enema is retained a subsequent injection of 2 to 3 pints of hypertonic salt solution should be given to precipitate the silver.

Smyly (1930) has reported good results with Dakin's weaker hyperchlorite solution. He begins with 25 per cent Dakin's solution injecting 300 to 500 cc. per rectum thrice daily. The concentration is increased according to the patient's tolerance up to the full strength of Dakin's solution.

Manson Bahr (1939) has found eusol the most efficient preparation but unless it is given highly diluted it is apt to be too irritating and cannot be tolerated by the patient. He begins treatment by the injection of half a pint (280 cc.) in a dilution of one part Budge's eusol to 9 parts of water, the enema being retained as long as possible. The strength of the solution is gradually increased until equal quantities of eusol and water are used. The treatment is frequently painful.

Tannic acid has been used especially on account of its astringent effect in cases where blood persists. Forty to 60 gr. to a pint of hot water have been employed. In all instances rectal injections should be preceded by a cleansing enema and it is essential for the best success that the solution should reach as high in the colon as the disease process exists.

**Vaccines.**—The value of vaccines in treatment is doubtful. Smyly reports that autogenous vaccines are sometimes effective in chronic cases. Fletcher, however, in the treatment by means of autogenous vaccines of carriers who still had lesions in the intestine, was unable to rid the intestinal tract of the dysentery organisms and found such treatment to be of little value.

**Bacteriophage** has been recommended for treatment by several observers. However in a number of instances where the results have been compared with controls not so treated, no value has been observed. [Riding (1930), Taylor and his associates (1930) and Kessel and Rose (1933)]. Kessel observed 68 cases, 35 who received bacteriophage by mouth in addition to symptomatic treatment and 33 who did not receive bacteriophage. Deaths and length of time in hospital were approximately the same in both groups. Gantenberg, in an epidemic of bacillary dysentery which followed the German invasion of Poland in September 1939, reported that the trials with bacteriophage (Polyphagin Behring) were on the whole inconclusive and unsatisfactory. Compton (1940) has recommended it in Cairo where its use is said to have become popular. Maj. Gen. H. Marrian Perry (1940) has stated that bacteriophage is neither advocated nor employed by the military forces in Egypt. Boyd and Portnoy (1944) have reviewed the subject and conducted important experiments in the field among German prisoners of war in the Middle East. In the prisoner of war camps selected for trial two separate restricted comparable communities were created by random grouping of cages into two series. Dysentery cases from one series received bacteriophage treatment, those from the other did not. A separate cage was set aside for a small experiment in prophylaxis. According to the

**Acidosis**—Symptoms of acidosis, which are sometimes superimposed on anhydraemia especially in children, must be watched for and treated with bicarbonate of soda either by mouth or intravenously. The intravenous injections of 1 per cent salt solution and 0.5 per cent glucose or of 0.5 per cent sodium chloride and 0.5 to 1.0 per cent sodium bicarbonate solution have been recommended in cases where adrenal insufficiency is suspected. Injections of adrenalin or of eucortine have also been employed and in the treatment of *ekiru* (the infectious diarrhoea of infants in Japan) Kawati reports subcutaneous injection of adrenalin 0.3 to 0.5 cc of a 1:1000 solution is an effective remedy.

**Relief of Pain**—Abdominal and griping pains are often relieved by hot fomentations, turpentine stupes and hot water bottles. Tenesmus and dysuria are best treated by hypodermic injections of morphine. Washing out the rectum with a pint of very hot water and subsequent introduction of suppositories of morphia and cocaine also frequently give relief. Vomiting and hiccough which are generally serious symptoms may be treated by hot stupes to the abdomen and the patient given ice to suck and small quantities of champagne or brandy.

**Other Drugs**—For checking diarrhoea and with the idea of eliminating the dysentery toxins in the intestine other drugs have been extensively employed. A mixture of animal charcoal and kayolin (*Bolus alba*) has been employed in doses of  $1\frac{1}{2}$  oz of each. A preparation known as colloidal kayolin or kaylenol has been recommended recently. In subacute cases isogel, a granular preparation of agar given in gram doses has been found useful in solidifying the stools and checking the diarrhoea. Bismuth has been employed for many years in drachm doses (3.8 gm) every 3 hours during the stage of diarrhoea. It is more suitable for mild and subacute cases. The carbonate or salicylate should be used but not the subnitrate which in large doses may liberate poisonous products. Intestinal disinfectants have also been employed and salol and rivinol have been particularly recommended for mass treatment during large outbreaks. The dose of rivinol advised for adults is 50 mgm in pills 3 times a day with correspondingly smaller doses for children. Salol is given in doses of 5–15 gr (0.3–1 gm) in cachets or suspension.

**Treatment of Chronic Cases**—Chiniofon (yatren) by the mouth and in rectal injections has also been recommended especially in subacute and chronic cases. 0.5 gm twice daily by mouth and 3 to 5 gm in 50 cc water by rectum. Colonic irrigations have been extensively employed in chronic cases. Cleansing enemata of 2 pts of hot saline solution or  $1\frac{1}{2}$  pints of 2 per cent sodium bicarbonate solution may be first employed.

C. F. Martin has employed silver nitrate 1 to 5 per cent in colonic injections while Rogers has employed albargin (silver gelatose) 20 gr to 1 pt of normal saline solution (0.5 per cent solution). Argvrol 40 gr to 1 pt of normal saline or 0.5 per cent solution has been extensively used. Silver nitrate must only be applied after the very acute symptoms have subsided. The solution should be given at 110° C and not retained for more than 1 hour. The silver compounds are only soluble in cold solu-

the writer's experience mild cases require no antiserum. They usually recover irrespective of such treatment and the most severe cases succumb in spite of treatment with serum.

Talbot (1937) with reference to the treatment of the disease in children says that no satisfactory specific serum is yet available for the infection. Only polyvalent serum is practicable as time is required for the bacteriological diagnosis.

The probability of serum sickness must be considered. It has been suggested to prevent or alleviate this to give calcium lactate 10 grains (0.64 gm) 3 times a day for 1 day before the administration of the serum and for at least 7 days following. But in the very acute cases it may not be regarded as wise to delay the serum until 24 hours after the first dose of calcium lactate.

The usual precautions against anaphylactic shock must be observed if the serum is to be given intravenously.

**Dosage**—Shiga formerly recommended a dose of 10 cc for a mild case or injections of 10 cc at intervals of 10 hours for cases of medium severity while in very toxic cases he used 60 cc in 3 daily doses of 20 cc each. The dosage of 20 cc formerly advocated is now considered by many to be too small for adults. Manson, Bahr and Chopra (1936) have employed 60 to 80 cc in adults. The dose may be repeated every 36 hours should the serious symptoms persist. Smyly recommends 2 daily injections of 40 to 80 cc. The doses of 100 cc or more which have nevertheless been employed are considered too large by some clinicians. A polyvalent serum should be employed and should be given as early as possible in the course of the infection. It may be given subcutaneously, intramuscularly or intravenously. All aseptic precautions must be rigidly observed and the serum should be heated to a little over body temperature by placing the vial in water heated to about 100 F (43.2 C) for about 5 minutes. The disadvantages of the subcutaneous method are that it is painful and the pain may last several days due to the distention and absorption of tissue produced by the large amount of serum injected. Also the serum is slowly absorbed. The points recommended for the injection have been especially the flank and the tissues over the lower part of the abdomen. Following the injection of the serum a general reaction is usually noted after about 12 hours and there may be flushing of the face and rise of temperature, increased pulse rate, increasing abdominal pain and often an increase in the number of the stools. But in the majority of cases on the following day the patient feels improved as regards pain and the effects of the toxæmia. The restoration of the normal bowel functions however takes place more slowly. If there is no improvement after 3 or 4 days evidently the serum is not proving effective.

Waller (1919) and Klein (1919) treated over 1300 cases in soldiers by subcutaneous injections of serum during the World War. Large doses of 120 to 140 cc were said to be most beneficial. The amount being given in 3 injections at 8 hour intervals. After the 7th day they found the serum had less effect. By this time the patient was regarded as either very likely to die or the stage of recovery had begun.

The intramuscular route causes much less pain and local disturbance. The sites recommended are the glutei muscles or the adductor group in the thigh. In the latter case care must be taken to avoid the femoral artery.

Especially in the fulminating form the serum should be injected into a vein. Either the medium basilic or cephalic veins at the elbow are the most suitable. Tainton (1921) and Wilcox after preliminary cleansing of the colon with a high enema have suggested high enema of serum 30-80 cc.



statements of German medical officers the standard treatment for bacillary dysentery in the forward troops of the German Army in Africa was Ruhr Bakteriophagen Polyvalent, 'Behringwerke' which carries the Bayer trade mark. It is put up in glass bottles in volumes varying from 50-500 cc. Large quantities of it were captured by the British during the Axis retreat from El Alamein. Boyd and Portnoy report no prophylactic action was found to result from the 3 day administration of the bacteriophage. The incidence of dysentery in the community treated with bacteriophage at the first sign of diarrhea was no different from that in the control community. Neither the severity nor the duration of the attack in the bacteriophage treated group was dramatically less than in the controls. Dysentery bacilli were recovered from the stools after the bowel had been exposed for as long as 4 days to the action of bacteriophage. This important article should be read in entirety.

*Surgical intervention* should only be considered in chronic cases where all medical measures have failed and in special instances where the condition of the patient is such as to suggest that there is some hope that he will be benefitted by the operation. Appendicostomy has been recommended and can frequently be performed with local anaesthesia. A small catheter may then be inserted into the caecum by means of which flushing out of the large bowel with hypertonic saline solution can be obtained. If it is deemed advisable to put the large bowel completely at rest valvular caecostomy or ileostomy may be performed.

Manson Bahr reports a case of an ex soldier aged 30 years who after suffering with severe chronic bacillary dysentery for 3 years underwent appendicostomy. This however proved a failure as did also bowel lavage with eusol solution. Valvular caecostomy was performed a year later and the bowel subsequently flushed out daily with hypertonic saline solution. This treatment proved more successful in that the patient when last heard from was in better health and physical condition but it had been impossible to close the caecostomy opening on account of the destructive process present in the large intestine and he still passed quantities of blood and mucus per rectum daily.

In other reported instances of caecostomy and ileostomy closure of the wound has been recorded as impracticable particularly on account of the stenosis contraction that has occurred in the ulcerated bowel which has not been in natural use for several months. For this reason terminal ileostomy which will enable the bowel to have complete rest is advocated by some. In such cases as Homans (1935) points out when proper care of the ileoanus is carried out and the movements become less frequent and irritating life is bearable and even may be enjoyable. However restoration of the natural passage is almost never possible. The disease does not always subside even in the functionless colon.

**Serum**—Anti dysentery serum has been employed for 40 years in the treatment of bacillary dysentery but there is by no means universal agreement as to the exact benefits to be derived from it. It has had a wide usage in many parts of the world. Manson Bahr who believes it is of value, nevertheless emphasizes that the serum must be used judiciously and with circumspection for it is essential only in very acute cases. In

spread the disease and a number of observers believe that the patient is the most important source of infection since the discharge may contain large numbers of bacilli in the first stages of the disease. Bacilli may continue to be present for from 4 to 5 weeks and in chronic cases occasionally for much longer periods. Disinfection of their stools is therefore important.

Kolmer (1923) has suggested the treatment of carriers by vaccination but Fletcher and others have found autogenous vaccines of little value in ridding the intestinal tract of the dysentery organism.

**Prophylactic Inoculation**—Protective inoculation has been employed for many years. Direct injection of killed cultures of the dysentery organisms particularly of the nonfermenting marine strains caused severe local and systemic reactions and therefore their use for prophylaxis has been largely abandoned. For these reasons the use of sensitized vaccine and of the addition of immune serum to the prophylactic was suggested in both Japan and Europe.

Gibson prepared a prophylactic issued in two vials consisting of the killed organisms in one and immune serum in the other. These were mixed in the syringe at the time of the injection. A locally painful induration usually followed the injection but the constitutional reactions were said to be milder than when the vaccine without serum was used. This method was used during the World War but its definite value was not generally conceded. However Dudgeon (1909) in Macedonia observed 67 cases of dysentery in 2096 non-inoculated individuals and only 14 cases of dysentery in 1147 individuals who were inoculated.

In Germany and Austria a sensitized dysentery vaccine *Bohches dysbakteria* has been employed and it was said that 100,000 people have been inoculated with it without deleterious effect. Its definite value however has not been conclusively demonstrated though Schittenhelm (1918) and Schelenz have reported favorably on its use as well as Bochncke and Elkeles.

Various other methods for minimizing the reaction following dysentery vaccines have been employed and the use of cultures killed by eusol has been advocated. Dean and Adamson (1916) found that 1:1000 eu sol was the minimum amount of concentration necessary to render the vaccine at all. Dudgeon (1909) reported that his experience with eusol treated dysentery vaccine was quite unsatisfactory. Perry and Coppinger (1925) reported that vaccines prepared from anaerobic cultures were much less toxic than those prepared from cultures grown in an aerobic manner. Others have suggested that formalized heated suspensions of dysentery bacilli cause far less local reaction.

Shiga's results of some 10,000 cases in which subcutaneous vaccinations with killed cultures and antiserum were given showed on the whole slight reduction in morbidity though there was a lowered mortality. Kauntze (1917) inoculated 50,000 porters in East Africa with 1-4 cc of a polyvalent dysentery vaccine. No improvement in the death rate from bacillary dysentery took place. Otto (1909) who has manufactured vaccine for dysentery at Frankfurt for many years has reported that he was able by its use to curb an epidemic affecting 3500 men at a military training post.

Edwards (1913), Dubois (1913) and Fraser (1913—unpublished) have made preliminary tests of a toxoid from *Shiga's* bacilli which give some evidence of the protective effect of bacteriophage in both animals and human beings in that the serum of inoculated individual 10 days after injection contained measurable amounts of specific antitoxin.

**Oral Vaccination**—Beddard from his studies of local immunity in infectious disease developed the bile vaccine method of giving the vaccine orally. He recommended to give before breakfast on 3 successive days

Many reports of the value of the serum are to be found in the early literature. Good results have been reported especially by Shiga in Japan, Rosenthal in Moscow, Ruffer and Willmore at El Tor, Kraus in Germany and Manson Bahr in Fiji. Serum was used extensively among the British troops during the World War. Graham at Salonica reports that the mortality was only 1 per cent in 200 average and severe cases treated with the serum. However, the general mortality among the British troops was only about 1.7 per cent. Fletcher and Jepps (1934) treated 246 Asiatics in Malaya by the intramuscular and intravenous routes. The results as compared with their control series were not striking. They thought the apparent failure of the serum treatment was not due to insufficient dosage or other such circumstances but to the exhausted and ill-nourished condition of the patients.

Smyly (1937) reports a series of 152 cases treated at the Peking Union Medical College Hospital, 79 were treated with serum. The apparent effect on these was good in 51 per cent, moderate in 25 per cent and nil in 24 per cent.

Acton and Knowles have reported that in India the serum is of value only in Shiga infections. Knauer (1936) reported that in the treatment of children with serum there was a diminution in the mortality rate. However, Talbot (1936) does not regard the serum treatment as efficient in children.

#### PROPHYLAXIS

The question of prophylaxis should include careful attention to personal hygiene. In institutions especially the washing of the hands of inmates before meals should be required. Nurses and attendants of dysentery patients should be informed of the danger they run of contracting the disease and should wear gloves when handling the patients. Care must be taken in handling rectal tubes employed in treatment and they must be carefully disinfected immediately after use. A 5 per cent solution of liquor cresolis compositus may be employed for this purpose. Special precautions must be taken against articles of food becoming contaminated either on the table or in the kitchen. Fruit, vegetables and milk are especially liable to become contaminated from the excrement of infested house flies. Water and milk should be carefully sterilized preferably by boiling, since many epidemics have occurred from infected water and a number from infected milk. The disinfection of the faeces is necessary and those passed must also be protected against the entry of flies. For disinfection of faeces one can use an equal portion of liquor cresolis compositus to a similar amount of stool, leaving the disinfectant to act at least one hour before emptying the receptacle. Bedding and soiled clothes can be disinfected in a 2½ per cent solution of this compound. Seats of toilets especially in institutions should be sterilized. Fly transmission from faeces especially in latrines and then to food is of the utmost importance in originating many outbreaks. The latrines must be made fly proof and all possible measures taken to exterminate the flies. Breeding grounds of the fly especially in manure heaps and in latrines must be destroyed.

On account of the infectious nature of the patients it is best in hospitals to care for them in special wards. Dysentery carriers may also

fever. This group includes *S. paratyphi* (paratyphoid A), *S. schottmulleri* (Paratyphoid B), *S. enteritidis* (Gartner) and *S. aertrycke* and the less important but closely allied organisms *S. sussexensis* and *S. morganii*.

These organisms are alike morphologically and culturally on ordinary media. They may be differentiated roughly from the colon group by their inability to ferment lactose and saccharose and from the typhoid and dysentery groups by their ability to ferment dextrose and mannite with gas production. The individual types react alike on a slant of Russells double sugar agar producing acid and gas in the butt and no change on the surface of the slant. For other biochemical reactions see the accompanying table p. 584.

#### PARATYPHOID FEVER AND FOOD POISONING

**Paratyphoid bacilli** (Achard and Bensaude 1896; Schottmüller 1901).—Paratyphoid fever, which bears a close resemblance to typhoid fever clinically, may be caused by *S. paratyphi* (A) or *S. schottmulleri* (B).

*Salmonella paratyphi* (paratyphoid A) is distinguished from other members of the *Salmonella* group by its ability to ferment xylose and to produce a brownish discoloration on lead acetate agar. This group is serologically relatively homogeneous.

*Salmonella schottmulleri* (paratyphoid B) produces acid and gas from xylose, discolors lead acetate agar and eventually renders milk strongly alkaline. Strains in this group vary in their antigenic properties and may be very difficult to classify.

Both organisms may cause a clinical picture indistinguishable from that of typhoid fever, although the symptoms are apt to be milder. They are present in the blood in the early stages and later appear in the faeces and sometimes in the urine. Agglutinins develop and the diagnosis may be made by agglutination and agglutinin absorption tests. A great many clinical types of paratyphoid infections occur, since there is as a rule less tendency for the bacteria to localize than for typhoid bacilli. Among these have been noted a dysenteric type, a nephritic type, a rheumatic type and an influenza type. Some cases of infectious jaundice have been attributed to paratyphoid infection and also various local infections such as pyelitis. *Paratyphoid B* may cause symptoms resembling those of meat poisoning. It is more pathogenic for animals than is the typhoid bacillus. The development of antibodies in man and in animals is much less marked than that which occurs in typhoid. Infection is transmitted in the same ways as is typhoid and some of the cases become chronic carriers.

Immunization with vaccines may be obtained as in typhoid fever.

**Laboratory Diagnosis**.—The same methods are used as in typhoid fever. Precise identification of the organisms is sometimes difficult even by agglutinin absorption tests, especially in the case of paratyphoid B, which may be diphasic. The flagellar antigen of a given strain may occur in 2 phases: a specific phase in which it is agglutinated only by a strictly homologous antiserum and a non-specific or group phase in which it is also agglutinated by antisera produced by the injection of the H antigen of other (heterologous) types. For exact identification of the organism it is necessary to test the agglutinability of the flagellar and somatic antigens separately. If the flagellar antigen is in the group phase it is essential that the culture be dissociated into the specific phase if necessary by growing it in media containing a group antiserum antagonistic to the heterologous elements of the group antigen.

**Antigenic Structure**.—The antigens of the *Salmonella* group have been studied in detail, especially by White and by Kauffmann, and the classification proposed by the latter investigator was recommended for general adoption by the *Salmonella* subcommittee of the International Society of Microbiology 1934. Thus far more than 40 types differing in their antigenic structure have been described. The type name is usually that of the locality at which the strain was first isolated.

a tablet containing 20 cgm of dessicated bile, followed by a dose of 100 milliards of dysentery bacilli killed by heat, the same treatment to be given on 2 further consecutive days

Since this suggestion by Besredka (1919) human vaccination by mouth with killed cultures of dysentery bacilli have been studied or employed by Kanai (1921) Japan Nicolle and Conseil (1922) Tunis Anglade (1924) and Pascal (1924) France Antonovsky (1924) Russia Gauthier (1924) and Seyfarth (1925) Greece All these observers have reported favorable results Vaz and Araujo (1929) have reported on the value of this method both for prophylaxis and treatment and Pergher and Van Riel have used a preparation known as anavaccine among employees in Central Africa and found the results favorable if given every six months Enlows (1925) has shown that immunity in rabbits could be obtained by oral vaccination in about 50 per cent of the animals Tanabe (1932) in the immunization of the Japanese army has also reported favorable results by the oral administration of anti-dysenteric tablets containing dried dysentery bacilli Iguchi (1932) used the oral vaccine on 130 000 school children during 2 successive years and reported that the morbidity rate from dysentery was reduced by half On the other hand Fulton and Berry (1927) who tried oral vaccine on children under 2 years of age in the United States obtained unsatisfactory results They employed a vaccine containing 400 000 000 each of 5 different strains A total of two thousand million per cc was given every month in milk on 3 successive days to 107 children leaving 397 untreated infants as controls The frequency of bacillary dysentery in the 2 groups was subsequently identical Walker and Watts (1930) also found that an oral bile vaccine prepared in Paris failed entirely as a prophylactic against bacillary dysentery

Prigge (1940) who formerly worked upon the preparation of diphtheria vaccine has prepared a dysentery vaccine consisting of a mixture of bacterial exotoxin with greater neural effects and endotoxin which is claimed to have a special action on the intestine It is reported that in animal experiments excellent results have been obtained and the first clinical observations are also said to be favorable

The entire subject of oral vaccine in dysentery must still be regarded as in the experimental stage Manson Bahr (1939) writes that the literature on the subject is in such a state of confusion that it almost defies analysis

Zusser and Bayne Jones (1939) believe that the available evidence does not warrant recommendation of the use of dysentery vaccines as a general prophylactic measure and in this opinion the writer concurs

## DISYENTERIC SYMPTOMS AND GASTRO INTESTINAL DISTURBANCES FROM FOOD POISONING

Gastro enteric disturbances and symptoms of dysentery also frequently follow bacterial infection of food or drink with organisms of the *Salmonella* group Kessel and his associates (1936) in studies carried out in California observed 246 cases in which organisms of the *Salmonella* group were found 72 per cent of these had acute dysenteric symptoms 16 per cent were diagnosed as chronic colitis and 12 per cent had no subjective symptoms Botulism which results from food poisoning with *Bacillus botulinus* a spore bearing organism does not give rise to gastro enteric symptoms Stitt Clough and Clough (1938) have summarized the classification of this enteritidis group and the disturbances to which they give rise

### SALMONELLA OR PARATYPHOID—ENTERITIDIS GROUP

This is a heterogeneous group of organisms some of which cause various gastrointestinal disorders and occasionally a disease resembling typhoid

The O antigens of which 13 have been distinguished are designated by arbitrarily chosen Roman numerals. The flagellar antigens in the specific phase of which 28 have been identified are designated by letters (a to z, z<sub>1</sub> etc.) and those in the group phase by Arabic numerals (1 to 6). The

## ANTIGENIC STRUCTURE OF SALMONELLA

Group	Type of organism	Somatic O antigen	Flagellar H antigen	
			Specific phase	Group phase
A	S paratyphi (Paratyphoid A) Senftenberg	I II I III	a gs	
B	S schottmülleri (Paratyphoid B)	IV V	b	1 2
	aertrycke (typhi murium)		i	1 2 3
	Stanley		d	1 2
	Reading	IV	eh	1 4 5
	Brandenburg		enlv	
C	S suispestifer American type	VI VII	c	1 3 4 5
	suispestifer European type			1 3 4 5
	Thompson		k	1 3 4 5
	Potsdam	VI VIII	enlv	
	Newport		eh	1 2 3
	Newport r Puerto Rico			1 2 3
	Newport var kottbus		eh	1 3 4 5
D	E typhosa	IX	d	
	S enteritidis		gom	
	enteritidis var Dublin		gi	
	enteritidis var Moscow		goq	
	sendai		a	4 5
	Panama		lv	1 3 4 5
	gallinarum (non motile)			

antigenic structure of a strain is usually determined by subjecting suitable suspensions of the organism to various immune sera from which all or most of the agglutinins except that corresponding to the antigen to be investigated have been removed by absorption with suitably chosen strains. Each type has usually 2 or 3 different O antigens in the specific phase, 1 to 4 H antigens in the group phase, 2 to 4 (other) H antigens. To identify a given type the culture must be in a smooth state; the organism must be examined in both the group phase and the specific phase, and it may be necessary to demonstrate the presence of 8 different antigens.

Imp r t n t n p b n g g m n g t t s n l b l l b d n t h l n t n b y o m m t t f t h t y t A m n a b t l g t a s g v n b y B g y	K t i p	C i t n	L i b B i k	I n d 1	V o g a p k	C i o s e	I a t	S c h s	M a l t	M n t e	B i t	S t a n t	A y l o s e	M n e	D i c t o l	A d m i t	G l y c e r o l	N e t s	H s t o n o f
E b n b h (B o l m m u )	X	-	AC	X	-	AG	AG	O	AG	AG	AG	A			AG	OG		X	-
E b n b h m m u n (B l m m u n )	X	-	AC	X	-	AG	AG	AG	AG	AG	AG	A			AG	OG		X	-
E b n b h t l g (B l g )	X	-	AC	X	-	AG	AG	AG	AG	AG	AG	A			AG	OG		X	-
E b n b h t l g (B l g )	X	-	AC	X	-	AG	AG	AG	AG	AG	AG	A			AG	OG		X	-
P t u l g n (B p t )	X	X	ACP	X	X	AG	AG	AG	AG	AG	AG	Alk	AG					X	-
S l m l l t r i d i s (G r n b l l u )	X	-	Alk	-	-	AG		AG	AG	O	AG	Alk	AG	O				X	-
S l m l l c h t m l l n (B p t y p h o s u s B )	X	-	Alk	-	-	AG	O	O	AG	AG	AG	Alk	AG	AG	AG			X	-
S l m l l p t y p h u (B p t y p h A )	X	-	Alk	-	-	AG	O	O	AG	AG	AG	Alk	AG	AG	AG			X	-
S l m l l r t k (B r t r y k )	X	-	Alk	-	-	AG	O	O	AG	AG	AG	Alk	O					X	-
S a l m n l l p t f (B a l l f b g b l )	X	-	Alk	X	-	AG	O	O	AG	AG	AG	Alk	AG	AG	AG			X	-
S l m l l m g n (B m g )	X	-	Alk	-	-	AG	O	O	AG	AG	AG	Alk	AG	O				X	-
E b r t h e l l t y p h (B t y p h )	X	-	Alk	X	-	AG	O	O	AG	AG	AG	Alk	O					X	-
S h g l l a d y t n (B d y n t n S h g )	X	-	Alk	-	-	A	O	O	A	A	A	Alk	AG	AG				X	-
S h g l l p d y t n (H )	-	-	Alk	-	-	A	O	O	O	O	A	Alk	O					-	-
S h g l l p d y n t n (F )	-	-	Alk	X	-	A	O	O	O	A	A	Alk	O					-	-
S h g l l p d y n t n (S e n g )	-	-	Alk	X	-	A	O	O	A	A	A	Alk	O					-	-
S h g l l p d y t n (S )	-	-	Alk	X	-	A	O	O	A	A	A	Alk	O					-	-
A l l g n f l (B f l l l l g )	X	-	Alk	-	-	O	O	O	O	O	O	Alk	O					X	-
B r u l l b t u (B A b r t )	X	-	Alk	-	-	O	O	O	O	O	O	Alk	O					X	-
B r u l l m i t (B m l t n )	X	-	Alk	-	-	O	O	O	O	O	O	Alk	O					X	-

The O antigens of which 13 have been distinguished are designated by arbitrarily chosen Roman numerals. The flagellar antigens in the specific phase of which 28 have been identified are designated by letters (a to z, z<sub>2</sub> etc.) and those in the group phase by Arabic numerals (1 to 6). The

## ANTIGENIC STRUCTURE OF SALMONELLA

Group	Type of organism	Somatic O antigen	Flagellar H antigen	
			Specific phase	Group phase
A	<i>S. paratyphi</i> (Paratyphoid A) Serflinberg	I II	a	
		I III	ga	
B	<i>S. schottmüller</i> (Paratyphoid B) aertrycke (typhus murium) Stanley Reading Brandenburg	IV V	b	1 2
			i	1 2 3
			d	1 2
		IV	ch	1 4 5
			enlv	
C	<i>S. suispestifer</i> American type <i>suispestifer</i> European type  Thompson Potsdam Newport Newport var. Puerto Rico Newport var. kottbus	VI VII	e	1 3 4 5
				1 3 4 5
		VI VII	k	3 4 5
			enlv	
		VI VIII	eh	1 3
				1 2 3
			eh	3 4 5
D	<i>E. typhosa</i> <i>S. enteritidis</i> enteritidis var. Dublin enteritidis var. Moscow sendai P. nama gallinarum (non motile)	IX	d	
			gom	
			gp	
			goq	
			a	1 4 5
			lv	1 3 4 5

antigenic structure of a strain is usually determined by subjecting suitable suspensions of the organism to various immune sera from which all or most of the agglutinins except that corresponding to the antigen to be investigated, have been removed by absorption with suitably chosen strains. Each type has usually 2 or 3 different O antigens in the specific phase 1 to 4 H antigens and in the group phase 2 to 4 (other) H antigens. To identify a given type the culture must be in a smooth state, the organism must be examined in both the group phase and the specific phase and it may be necessary to demonstrate the presence of 8 different antigens.





known however that the disease is caused by a filtrable virus and that although this organism is constantly present and may be isolated from the blood it is only a secondary invader. It has been reported as the cause of epidemics of food poisoning in man although in some of these cases it was probably confused with *S. aertrycke*. It has also been isolated from sporadic cases of a severe general infection in man (most frequently in children) which resembles typhoid fever clinically. (Reviewed by Harvey 1937.) Identification of this organism require precise agglutination and agglutinin absorption tests. The H antigen of the European type is monophasic (group phase) whereas that of the American type is diphasic (see table). According to Kottner and Zepp it can also be differentiated by its inability to ferment arabinose, trehalose and inositol.

*Salmonella morganii* has been reported as the cause of certain cases of mild enteritis. It produces a very slight amount of gas in glucose only and produces indole. It does not cause any primary acidity in litmus milk.

Other closely related organisms with slight antigenic differences have been described in various epidemics.

	Botulism	Food infections
Cause	<i>Botulinus</i> toxin	Bacilli of the salmonella group
Fever	Not characteristic temperature usually subnormal	Characteristic acute
Occurring	Mainly in winter	Mainly in summer
Associated with	Preserved foods	Fresh foods or freshly contaminated foods usually meat or milk
Condition of bowels	Constipation rarely diarrhoea	Diarrhoea offensive
Visual disturbances	Double vision ptosis of lids	Absent
Abdominal pain	Absent	Present
Onset	Usually gradual	Sudden
Incubation period	Variable usually from twelve hours to several days	Short usually from six to twelve hours
Throat	Swallowing difficult	Normal
Treatment	Antitoxin	Systemic
Mortality	From 60 to 70%	From 1 to 2%

*Laboratory diagnosis in cases of food poisoning* due to these organisms depends chiefly upon their isolation from the stools by plating methods. Occasionally they may be demonstrated in blood cultures. During convalescence agglutinins may be formed which can be tested against known strains of these bacteria. Cultures from the infected food should be made when possible.

*Potomac Poisoning.*—This term is based upon a misconception. The split products of protein putrefaction have not been demonstrated to have a toxic effect when ingested. This diagnosis has often been applied to disturbances resulting from infection with *Salmonella dysenteriae* or colon bacilli. Botulism must also be considered. Anaphylactic reactions to certain food stuffs may cause similar gastrointestinal disturbances.

An organism may be assumed to be in the group phase if it is agglutinated by an H agglutinating serum for *S. suspestifer*, European type since the latter occurs only in the group phase

The table on page 585 illustrates the complexity of structure and the apparently haphazard way in which the different O and H antigens are combined in some of the types (For a full discussion see Topley and Wilson Bacteriology 1937) (See also Seligmann and Wassermann 1943)

*Salmonella enteritidis* (*B. enteritidis*) Gartner 1888—This organism has been isolated frequently from cases of gastroenteritis caused by the ingestion of meat from diseased animals or even of food contaminated by contact with the infected meat. The infection may be spread by the unclean handling of food by flies or even by the contamination of food with the faeces of mice or rats. This organism and *B. aertrycke* are particularly responsible for the outbreaks of food poisoning which have occurred in Germany, England and less commonly in the United States.

It closely resembles the paratyphoid B bacillus in its cultural reactions but can be differentiated from it by its ability to produce acid in tartrate media and by agglutination tests with immune serum.

In man *S. enteritidis* causes an acute gastroenteritis with symptoms of intoxication. Since *Salmonella* have been isolated from only 20 to 30 per cent of the cases of food poisoning, Savage (1929) suggested that the symptoms in some cases may have been due to endotoxins formed in the meat before ingestion. If the amount of toxin ingested is great symptoms occur shortly after ingestion. If the amount of toxin is small the symptoms may be delayed for one or two days. This toxin, unlike that of *B. botulinus* (*Cl. botulinum*), is not destroyed by boiling. The Gartner bacillus has been isolated in pure culture from the faeces in cases with high fever and marked intestinal derangement with fluid stools containing considerable blood. It is very pathogenic for laboratory animals producing a haemorrhagic enteritis and at times a septicaemia. In large outbreaks the symptoms often commence almost simultaneously among a number of the food consumers. The onset is usually sudden with abdominal pain and tenesmus, diarrhoea, nausea and usually vomiting and continuous vomiting usually denotes a serious prognosis. While in some instances the stools may contain considerable blood, blood and mucus in masses in the faeces are a rare occurrence.

*SALMONELLA AERTRYCKE* (*B. aertrycke*)—This organism frequently causes a similar gastroenteritis. It resembles the paratyphoid B bacillus even more closely than *S. enteritidis*, and is difficult to differentiate serologically, even by agglutinin absorption tests. Both are diphasic but in the specific phase they possess different flagellar antigens. *S. aertrycke* (identical with *B. typhimurium*, *B. pestis caviae* and *B. psittacosis*) is highly pathogenic for many laboratory animals and causes serious epidemics especially among mice and guinea pigs.

*S. suspestifer* (*B. suspestifer*)—This organism was isolated by Salmon and Smith from swine with hog cholera and was believed to be the etiological agent. It is now

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Burke and May have tabulated the differences in botulism and food infections as shown on p. 587

Staphylococci and streptococci have also been reported as the cause of outbreaks of food poisoning. Dack, Jordan and others in Chicago have reported that certain strains of staphylococci are able to give rise to toxic substances which may have a considerable degree of thermostability and result in the formation of bacterial toxins in the food prior to its consumption. The staphylococcus in Kelly and Dack's experiments was isolated from food which had caused an outbreak of food poisoning. While it grew best at 37°C it also grew later at temperatures as low as 8°C. It could also be cultivated in media containing 10 per cent salt. It penetrated rapidly into meat as well as into bread especially if the latter was moist. The usual symptoms of the outbreaks were nausea, vomiting and abdominal pain, the clinical symptoms of dysentery being usually not present. Kelly and Dack reported 17 epidemics due to staphylococci. A number of other reports have been made of severe diarrhoea caused by staphylococcus enterotoxin. It has been thought to be due to a preformed heat stable toxin in certain foods containing custard or cream fillings, sour milk and ham injected with curing fluid (tenderized ham) have also been regarded as a source. Certain haemolytic strains of staphylococcus have been regarded as the infecting organism in the food. Callender and Inmon (1937) have reported 2 other epidemics in the Panama Canal Department within a 12 month period, one due to the ingestion of contaminated bread pudding and the other to ham left standing at the tropical kitchen temperature 48 hours after boiling and then used to mince as part of the filling of stuffed eggs. Organisms of the Salmonella group also may give rise to thermostable toxic substances which are soluble in water and are precipitated by alcohol. The method of extracting these substances has been described by Raistrick and Topley who have shown that they are polysaccharides and when injected into rabbits cause fatal symptoms.

#### DYSENTERIES RESULTING FROM MECHANICAL IRRITANTS OR POISONOUS SUBSTANCES

Stitt has reported a form of poisoning which occurs in North China and gives rise to serious illness or death and is attended with marked abdominal pain and manifestations of dysentery caused by short lengths of bristles which are given mixed with the food.

Various irritant metallic poisons, as arsenic, antimony and mercury may also give rise to dysenteric symptoms. Callender has reported an outbreak in the United States Army caused by poisonous amounts of zinc and antimony in limeade which had been prepared in a galvanized iron pan. The onset of the disease was spread over 4 days and 80 per cent of the men who drank the limeade were affected. Poisoning with mercury may give rise to ulceration of the intestine and secondary infection with a virulent exudate in the stools. Intussusception frequently gives rise to symptoms of pain and tenesmus and vomiting with the passage of small, bloody rather than mucous sanguineous stools. A sausage shaped swelling may appear in the abdominal region. Dysenteric symptoms may likewise be present in the terminal stages of various chronic diseases, especially tuberculosis and cardiac infections. In cancer and syphilis of the rectum there may be a suspicion that the process is an ordinary dysenteric one. Also in chronic nephritis leading to uraemia symptoms of a marked catarrhal or even diphtheritic colitis may occur.

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carried there from India. During the epidemic of 1817 it again invaded China by the land route from India and extended all over Asia. It was first described from Japan in 1821 although an epidemic which devastated Tokyo in 1718 may have been cholera.

Five pandemics of appalling magnitude have occurred during the 19th century, spreading from India through Asia Minor, Egypt and Russia, and by 1830 reaching not only Central Europe but North and South America.

The great pandemic of cholera which started in India in 1817 extended all over Asia but did not invade Europe. The second great pandemic is of importance as being the first to invade Europe. It started in India in 1816 and advancing slowly reached Persia in 1819, extending thence by way of Astrakhan to Russia, Sweden, Northern Europe and England. By 1831 it had spread over the whole of Europe. In the same year 1831 it reached Canada and thence extended to Fort Dearborn where it infected the soldiers who subsequently carried the disease down the Mississippi valley. It was also introduced into New York and Boston and spread from there south and west so that by 1836 cholera was present in most parts of the United States not disappearing until 1838. It disappeared from Europe in 1839.

The next European outbreak of this pandemic lasted from 1846 to 1861 and was traced from India by way of land and sea, that by land following the caravan route by way of Persia and Russia and that by sea from Indian pilgrims going to Mecca and there causing the infection of Mohammedan pilgrims from Egypt and European Turkey. This pandemic reached the United States in 1848 starting at New Orleans and extending up the Mississippi valley. Central and South America and the West Indies were also invaded by the third pandemic. The fourth great pandemic invaded Europe by the usual routes and continued from 1863 to 1875. In 1861 it was carried by sea from Bombay to Arabia and Mecca and was then spread by the returning pilgrims throughout Egypt, Syria and the southern European ports to the East Coast of Africa. During the continuance of this pandemic there were 2 outbreaks in the United States, one in 1867 and another in 1873. That in 1873 when it was introduced into 3 widely separated parts of the country was the 1st appearance of an outbreak of cholera in the United States.

The fifth pandemic began in India in 1879, reaching Egypt and Europe in 1883 and affecting particularly in Europe the Mediterranean seaports of France, Spain and Italy. It was during this epidemic in 1883 that Koch working in Egypt discovered the cause of cholera, *Vibrio comma* (*Sp.illum cholerae*). However, as the epidemic in Alexandria soon subsided he proceeded to India where after a study of 42 cases of cholera and 28 autopsies he gave confirmatory evidence of the etiology of the disease (1884).

A very serious outbreak of cholera originated in 1891 in pilgrims from the delta of the Ganges attending a religious festival. It was spread by returning pilgrims and reached Europe in 1891. Almost a million deaths occurred in Russia. It was during this epidemic that cholera appeared with great virulence in Hamburg. In that city within 2 months there were nearly 17,000 cases and over 8,000 deaths. This outbreak gave opportunity for those careful studies as to the transmission of the disease as to be later referred to.

It is usual to recognize a sixth pandemic which began in 1902 and spread over India, China and the Philippines. The epidemic in the Philippines gave opportunities for special and original studies concerning the disease by American officers of the Army and Civil Government medical services. This pandemic continued to cause great mortality in Europe and from 1908 to 1910 there were reported some 71,000 cases and 26,000 deaths in Russia.



## Chapter XVII

### CHOLERA

#### DEFINITION

**Definition** —Cholera is an acute infectious disease characterized by a profuse and purging diarrhoea by vomiting muscular cramps, suppression of urine and collapse. It is caused by a bacterium *Vibrio cholerae* which is present in the intestines and in the rice-water like stools during the acute stage of the infection.

This organism multiplies especially in the small intestine and under going lysis liberates a toxin which is responsible for the desquamation of the epithelium of the mucosa and the initiation of the other manifestations of the disease. The clinical course is divided into the stage of evacuation, in which there is a great loss of fluid from the body through repeated profuse discharge of rice-water like stools and copious vomiting accompanied by very painful cramps of the muscles. These symptoms are followed by the algid or collapse stage with signs of failure of circulation and almost imperceptible pulse, hoarse whispering voice, cold, clammy skin, subnormal axillary temperature, shriveled and cyanotic extremities often associated with anuria. With the return of activity of the circulation and urinary secretion a stage of reaction supervenes.

#### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** —There are indefinite references to cholera in the early Greek literature. Thucydides 5th century B.C. (Book II Sec. 47-53-4) suffered from a disease he described as epidemic among the Athenians which caused great mortality. In his description of the symptoms he refers to the fact that with the contents of the bowels running out like pure water the patient must sink at last through asthenia due to this. McMillan (1914) translated the account given by Thucydides and regards it as the first recorded cholera epidemic. Although the word *χολέρα* (sometimes translated as flow of bile) is found in the writings of Hippocrates it is generally agreed it did not refer to the disease we now recognize as cholera. The older writers noted bilious discharges as characteristic of the malady they termed cholera which could not apply to the bile free rice-water discharges so characteristic in the disease that we now term cholera. Susruta in India in the 7th century A.D. described a disease in which there were diarrhoea and vomiting, stabbing pains, cyanosed lips and nails, with sinking in of the eyes and weak voice. Indeed it appears not improbable that cholera has been present in India from remotest antiquity. For centuries it has been known to be endemic in the delta of the Ganges and lower Bengal and from there it has spread from time to time in epidemic form not only all over India but to many other countries assuming on a number of occasions world wide pandemic characters. Detailed accounts of the presence of cholera in India were published from the 16th to 18th centuries when the Portuguese, English and French were carrying on their wars of conquest in India. These wars served in spreading the disease all over that country. It has been thought that true cholera did not exist in China until 1669 when it was

In a number of these instances this immunity is due to the geographical isolation and lack of communication with the endemic centers of the disease. However one should bear in mind that cases of cholera may sometimes appear very unexpectedly in a country in which the disease is not known to be present. One need only recall the sudden epidemic in Hamburg in 1892. At the time of its origin cholera was not supposed to be present in Germany. Moreover there is hardly an important country in the world which has not at one time or another been visited by cholera during some of its epidemic periods. That certain countries are free today is due particularly to quarantine and to the other sanitary measures which are taken with reference to the prevention of the disease.

### ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—The cholera vibrio *Vibrio cholerae* (*Spirillum cholerae*) the cause of the disease was discovered by Koch in 1883 and is a short curved organism which from its shape is often called the comma bacillus.

**Morphology**—Typically it is a small comma-shaped rod 1.5 by .3  $\mu$ . It frequently occurs in S shapes owing to the attachment of a pair of organisms at their ends and especially in old and avirulent cultures long threads showing a somewhat spiral appearance may be seen. In smears made from bits of mucus and cellular debris in the faeces the spirilla often resemble fish swimming parallel to one another in a stream. After prolonged artificial cultivation and occasionally in freshly isolated cultures rod forms coccoid and club shaped in solution forms are frequent. Ohno found that their development depends in part upon the reaction of the medium and suggested that transfers be made on media of varying pH to obtain the characteristic vibrio morphology. There is a single long terminal flagellum which imparts to the organism a very active scintillating or darting motility. It stains easily by ordinary methods and is Gram negative.

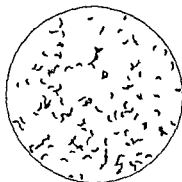


FIG. 146—Cholera vibrio (Killed by Wassermann)



FIG. 147—Involution form of the vibrio of Hilfer (Van Ermengem)

**Cultural Characteristics**—The *Vibrio cholerae* is strictly aerobic and grows readily upon ordinary culture media. The optimum reaction is pH 8.0–9.0. Growth is inhibited by a moderate acidity but will occur on media sufficiently alkaline to inhibit other species of bacteria. This tolerance for alkalinity facilitates their isolation from the faeces by special media. On agar the colonies are translucent bluish grey resembling somewhat those of the typhoid bacillus. On gelatin plates they are more characteristic and appear after 4 hours as small highly refractile whitish colonies which under the low power have a granular center with spinose margins and a surrounding zone of liquefaction. In gelatin slabs a turnip-shaped area of liquefaction appears at



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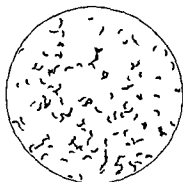


FIG 146—Cholera vibrio (Killendorn)



FIG 147—Involution form of the vibrio of Shiga (Van Ermengem)

**Cultural Characteristics**—The vibrio cholerae is actively aerobic and grows readily upon ordinary culture media. The optimum reaction is pH 8.0-9.0. Growth is inhibited by a moderate acidity but will occur on media sufficiently alkaline to inhibit other species of bacteria. This tolerance for alkalinity facilitates their isolation from the faeces by special media. On agar the colonies are translucent bluish grey resembling somewhat those of the typhoid bacillus. On gelatin plates they are more characteristic and appear after 4 hours as small highly refractile whitish colonies which under the low power have a granular center with spiny margins and a surrounding zone of liquefaction. In gelatin slabs a turnip shaped area of liquefaction appears at

the top of the puncture—the air bubble appearance. Coagulated blood serum is liquefied. Litmus milk is (usually) not acidified nor coagulated. On alkaline potato the growth is whitish and later changes to a brownish yellow or pinkish color. In broth or in Dunham's peptone solution growth is rapid and luxuriant especially at the surface and a pellicle is formed. In the latter medium indol is produced and the nitrates are reduced to nitrites. The cholera red reaction depends upon this fact and is due to the formation of nitroso indol. The test should be made by adding from 6 to 8 drops of concentrated  $H_2SO_4$  to a 24-48 hour old peptone solution culture of the organism to be tested. Each lot of peptone should be tested with a known cholera vibrio since certain preparations of peptone will not give the reaction. If the organism is a cholera vibrio both indol and nitroso body are produced and the violet pink coloration occurs, cholera red reaction.

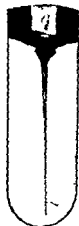


FIG 148—  
Vibrio of  
cholera. Stab  
culture 11 gel  
atin two days  
old (Fraenkel  
and Pfeiffer)

**Fermentation Reactions**—Glucose, maltose and saccharose are usually fermented. Acid is produced without gas. Xylose is not fermented. Most of the strains ferment mannite. Lactose is not fermented within the first 48 hours and many strains have no action on this subsequently. But slow or late fermentation of lactose has been reported. However the fermentation reactions do not suffice to differentiate the members of this group though Taylor Read and Pandit (1936) report that the fermentation of mannose is characteristic of typical *V. cholerae*.

Many believe that the typical cholera vibrio does not produce haemolysis on blood media although after several days growth there may be some chemical alteration or digestion of the medium around the colony which simulates a zone of haemolysis. VanLoghem has suggested that this may be due to haemodigestion. Cooked blood medium is cleared in the same way. If a filtrate from a broth culture is added to a suspension of red blood cells no haemolysis usually occurs. An exception to this rule is seen with the El Tor vibrio which was isolated from cases of diarrhoea, sometimes fatal in pilgrims at El Tor. This organism is actively haemolytic yet is agglutinated by cholera immune serum.

**Vibrio El Tor**—The relationship of the *Vibrio El Tor* to *Spirillum cholerae* has been a matter of controversy in bacteriology since 1905 when it was first found by Gotschlich at the quarantine station at El Tor in both sick and healthy pilgrims. The organism has been found in the complete absence of cholera in the region and it has hitherto sometimes been regarded as non pathogenic for man. Recently however DeMoor (1938) has reported an important outbreak in the Celebes in which from 47 patients with typical symptoms of acute cholera a vibrio of the El Tor type was isolated.

He identified this El Tor vibrio by applying agglutination tests of the nonspecific H agglutinin and that of the specific O agglutinin (Inaba and Ogawa types). These were positive as also was the Pfeiffer test. Haemolytic activity was shown for goat erythrocytes. About 400 strains were examined and they were identical. He considers the disease endemic and not an accidental importation. VanLoghem (1938) has studied 2 of these vibrio strains sent him by DeMoor. He found the organisms identical with the classical El Tor vibrio in all respects. As a result of his studies on haemolysis he concludes that the vibrios of Koch and of El Tor are different hence the Celebes outbreak is regarded as not identical with acute cholera. He proposes to call it *enteric choleraformis* although clinically the disease resembled cholera. Otten (1939) has examined this Celebes strain grown in broth and has found it to be definitely haemolytic but far less so than *Vibrio El Tor*. He believes also that *Vibrio cholerae* may give rise

to a haemolysin which is often less stable than that of *V. celbes* and as a rule is already absent in cultures of 3 days growth. In 1940 eight cases of El Tor *Vibrio* infection occurred in the Celebes and 5 of these were fatal.

*Haemolytic Power of Cholera Vibrios*—Ottén emphasizes that whether haemolysis is demonstrable or not depends considerably upon the method employed in making the tests. At least 3 factors are of paramount influence in the haemolytic process, namely the growth of the culture, the method of incubation of the mixture, and the way in which the blood is subjected to the action of haemolysis. He found that haemolysis subsides when, according to VanLoghem's method, blood is added first to the broth and then inoculated only after the blood has settled at the bottom of the tube, instead of adding blood to the already full grown, one-day culture. In the latter case, haemolysis usually occurs.

Both DeMoor and Ottén have observed the presence of haemolysis in one-day cultures in the majority of the cholera strains studied. However, they found that when they employed the medium blood ratio used by Greig, namely 1 cc. of the broth culture to 1 cc. of a 5 per cent blood suspension after continuous incubation at 37°C, they obtained no haemolysis. Ottén thinks this inhibitory action emanates from the blood itself. In vibrios with a weak haemolytic power, haemolysis was found to fail even when only twice the usual quantity of blood was used.

Kabeshima (1938) previously reported that over 90 per cent of his cholera strains showed haemolysis, and Jenevray and Bruneau (1938) reported that most of their cholera strains after 24 hours incubation at 37°C showed a discrete haemolysis, but this was sometimes irregular.

Mertens and Beuwkes (1940) experienced some difficulty in separating authentic cholera vibrios from the El Tor and Celebes strains by means of the haemolytic test. However, by extracting the 3 kinds of vibrios with acetone alcohol, they obtained a thermostable haemolytic agent from the El Tor and Celebes strains which was present only in small amount in two of their cholera strains. Secondly, by growing the 3 vibrio types on a synthetic glutamic acid medium to which goat red cells had been added, a complete differentiation was found possible. All their experiments were in accord in showing the identity of haemolytic properties of the Celebes and El Tor strains and their difference in this respect from the true cholera vibrios.

Goyle (1939) thought that the haemolysins were true exotoxins since they were thermolabile, antigenic and filterable.

*Cultural Variability*—Great variations in the characteristics of the colonies in morphology, motility and biochemical activity in different strains of *V. cholerae* have been reported. In 1913, Sato and Takaka described 2 different strains of cholera vibrios distinguishable by the behavior of the agglutination test. They named these types the Inland and Formosa types. A short time afterwards, Kabeshima classified a large number of cholera vibrios into 2 types: 1, the original type, and 2, the varied type. Nobecki later confirmed Kabeshima's results, but in addition he found a third type of vibrio which he named the middle type. This was agglutinated by both the serum of the original type and the varied type of Kabeshima. In medical literature today, the original strain is known as the Inaba type, the variant strain as the Ogawa type, and the middle type rarely employed as the Kojima type. Shousha (1923) examined 2 strains of a cholera vibrio, one a haemolytic strain, yielded 2 variants, S and R. The R variant was found to agglutinate spontaneously in physiological saline solution. The 2 variants were found to differ in cultural characteristics, agglutination with specific sera, and complement fixation. One was more pathogenic than the other. Baitenau (1926) dissociated the cultures of vibrios into 3 types of colonies: (1) a rugose, circumvalent type with a central nodule and thin zone and thickened edge; (2) a white ringed type composed of a dense center and a thin flare bordering zone; and (3) an opaque, round, hemispherical type with a regular surface. These colonies do not exactly correspond to the usual smooth, rough transformations. The organisms in the opaque colonies were non-motile. The morphology of the vibrios in the varying types of colonies were not strikingly different.

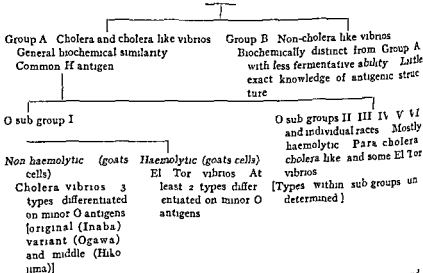
During the growth of cultures, he described coccoid, bacillary, long spindled and budding or branching forms. The writer has never observed budding or branching forms in any fresh virulent cultures of the cholera organism. However, Baitenau

clearly demonstrated that cholera vibrios do not lack the H or flagella antigen as Wel and Felix had assumed. The vibrio possesses only a single flagellum and hence carries only a small amount of flagella material. Baltenau obtained the flagella in concentration and showed that they were composed of the usual heat labile loose flocculating H antigen with specific properties. The bodies of the motile vibrios and the non motile forms contained the heat stable O group antigen. Immune serum prepared against organisms heated to 100 C for 2 hours contained only O agglutinins.

**Serological Relationships**—Recent studies especially those of Gardner and Venkatraman (1935) have shown that the antigenic structure of *V. comma* and related vibrios is very complex. The true cholera strains appear to constitute a relatively homogenous group. There are however numerous strains obtained from various other sources which are culturally and biochemically identical with *V. comma* and possess the same H (flagella) antigen but which have different O (somatic) antigens. They are therefore agglutinated by ordinary anti cholera serum which contains both H and O agglutinins. They think a diagnostic immune serum therefore should be prepared from an antigen from which the non specific H component has been removed. Some of the El Tor vibrios have been found to possess the specific O antigen of the true cholera group while others are related to it only through the H antigen.

Topley and Wilson (1936) have for purposes of convenience classified the cholera and cholera like vibrios into 2 groups A and B. Group A comprises organisms most of which produce acid without gas in glucose maltose mannitol and sucrose but not in dulcitol and which give the cholera red reaction. All organisms of Group A possess a common H antigen. The major O antigens on the other hand of which 6 have already been differentiated are much more specific and are used as a basis for the differentiation of Group A into sub groups. The true cholera vibrios all appear to fall into sub group I which also contains most of the El Tor strains. Sub groups II to VI contain organisms referred to as paracholera and cholera like that have been isolated from cases of choleraic diarrhoea or from water. Thus according to Gardner and Venkatraman the true cholera vibrio is a non haemolytic organism containing the specific O antigen of sub group I. Except by haemolysin production it is indistinguishable from El Tor vibrios containing the same O antigen. They have also described a non specific O antigen shared to a variable extent by all members of Group A.

### Vibrio



Modified from Topley and Wilson *Principles of Bacteriology and Immunity* 2nd edition 1936 Wm Wood & Co Baltimore

This classification refers to the antigenic structure of vibrios in the smooth state. The cholera vibrio is readily dissociated and a number of variant strains have been produced by various methods of cultivation.

Bruce White (1935) by exposing young smooth cultures to the homologous activated antisera has transformed them to the rough state and has proposed another classification of the vibrios in the rough state. Most of the organisms were lysed by this treatment but among the survivors there was a considerable proportion of the rough type and these were found to be stable on plating. By the further treatment of such R colonies with activated anti-R sera, races designated as  $\rho$  types were obtained and these in turn showed greatly reduced agglutinability with the anti-R sera. The R strains were serologically more generalized than the S strains in that serological differences between the latter were found to have disappeared.

Just as is the case with the Salmonella group, White found that the transformation of the smooth to the rough phase is accompanied by a loss of specific O antigen and the unmasking of the common rough antigen. In consequence of this many organisms that were antigenically diverse in the smooth state show a close similarity in the rough state.

White has also described the occurrence of alcohol-soluble protein antigens which he refers to as Q antigens. There is some reason to believe that these substances may play a part in the non-specific O agglutination of boiled vibrios described by Gardner and Venkatraman.

**Classification.**—The cholera vibrios are readily dissociated and a number of variant strains have been produced by various methods of cultivation. White (1936) later demonstrated that the S (smooth) cholera vibrio possesses serologically active polysaccharide or nonprotein carbohydrate-containing receptors which he terms C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, and C<sub>4</sub>. Of these C<sub>1</sub> the determinant of smooth type specificity is lost in roughening; exposing C<sub>2</sub> the characteristic rough polysaccharide. On degradation to the  $\rho$  form C<sub>2</sub> disappears but C<sub>3</sub> and C<sub>4</sub>—substances common in whole or in part to many vibrios—remain. On digestion of cholera vibrios with pepsin in a slightly acid medium C<sub>1</sub> and C<sub>2</sub> are brought into solution; C<sub>3</sub> and C<sub>4</sub> are not but may be liberated from the residue with alkali.

According to Linton et al. these changes which give rise to variants depend primarily upon a loss or chemical alteration of the specific carbohydrate. In one rough variant they found that both protein and carbohydrate constituents were altered.

Linton and Mitra (1938) examined the strains of White to determine possible differences between the S and R homologues with respect to chemical structure, electrophoresis and metabolism. They found the chemical structure and metabolic activity closely correlated and changes in their chemical structure were accompanied by changes in metabolism. The serological change brought about by treatment with antiserum or by other processes follows changes in chemical structure, metabolism and surface potential. After subjecting the organisms to electrophoresis the shift in the surface potential was regarded as the cause of serological distinction of the R derivatives from their S homologues and to partly account for the uniformity of serological behavior of the rough vibrio strains in general.

From an extended study of the chemical composition of the vibrios Linton (1938) has been able to classify them into 6 groups which correspond with their origin, biochemical activities and metabolism. Metabolism was found highest in Group I (vibrios from clinical cholera), somewhat less active in Groups II and V (from cholera cases or carriers) and Group VI cases from carriers. It was least active in the water vibrios of Group III. Group IV which contained vibrios from El Tor and from India was characterized by a rate of respiration equal to that of Group I and by the absence of aerobic glycolysis.

It was found that rough strains had a lower metabolism than smooth strains, nonagglutinable strains than agglutinable strains. The El Tor strains could be easily dis-



tinguished from vibrios from cases of cholera by chemical means but not by means of O agglutination (Gardner and Vankatraman). Nevertheless vibrios belonging to their chemical Group II from cases of cholera were found to contain the same polysaccharide as those of chemical Group III from water but the 2 were quite distinct in the agglutination test. They found 2 chemically and serologically distinct types among the cholera strains. The chief point of difference was that the strains from the early part of the epidemic contained a lipoid polysaccharide complex which was absent in strains obtained from the latter part of the epidemic in strains maintained for a long time in the laboratory and in water and carrier strains.

Linton Seal and Mitra (1939) have studied the phenomena of bacterial variation by means of single cell culture. In this way they succeeded in obtaining a new strain with different biochemical, cultural and serological characteristics and a different chemical structure from those which characterized the culture from which the original single cell was taken. The new strain was still however within the protein and polysaccharide frame work of the original and the capacity for transformation was therefore limited.

On the basis of further single cell experiments Linton found that the association of metabolic activity with chemical groups appeared to be reasonably constant and that it corresponded with changes of chemical structure in the course of variation. He suggests that an organism possessed of a common protein and polysaccharide can be transformed into one having a protein and polysaccharide of entirely different type. The resulting organisms are given the rank of strains or variants and it is pointed out that the original strain becomes 2 strains each having distinct chemical structure. At the same time the serological, biochemical and metabolic characteristics of the variant differ from those of the original and resemble those of the new group into which the variant now falls.

The metabolic technique has been applied to over 300 vibrio strains. The groupings are classified as (1) Chemical groups I and VI from cholera cases and contacts with protein I of a higher metabolism. (2) Chemical groups IV and V from carriers both in India and El Tor with Protein II, an intermediate metabolism. (3) Chemical group II from water vibrios with protein II, a low metabolism and a different polysaccharide from Groups IV and V.

If it can be shown that variations such as these can occur *not only* in the laboratory but also in nature in the field, an answer may perhaps be found to the question whether cholera cases arise only from previous cases and from contacts with such cases or whether the chronic carriers of Groups IV and V can sometimes spread epidemics.

It has been reported that, experimentally, variant types may be produced also by the action of various types of bacteriophage. These changes are ascribed by Morison, 1935, to alterations of the bacterial protein by hydrolysis. For a complete discussion of the chemistry and serology of the vibrios the reader is referred to the article of Linton (Bacteriological Reviews Dec 1940).

**Pathogenicity**—Feeding or subcutaneous injection of the cholera vibrio does not usually cause infection in adult animals. Koch however sometimes produced infection in guinea pigs by introducing the organisms together with alkali into the stomach and giving them opiates to inhibit intestinal peristalsis.

Intraperitoneal injection of virulent cultures into guinea pigs gives rise to a fatal peritonitis. However, when the organisms are injected into an immunized guinea pig or when a small amount of cholera immune serum is simultaneously injected, bacteriolysis takes place and the animal

recovers. Rabbits usually succumb from the intravenous injection of virulent cultures in doses of 2-4 mg but not of 1 mg (one half loop)

There have been a few instances where cholera has been caused in laboratory workers by the accidental ingestion of cholera cultures thus Orgel was infected from sucking up peritoneal fluid in doing Pfeiffer tests for bacteriolysis and died. In connection with other epidemiological studies regarding the disease and doubting the pathogenicity of *Vibrio cholerae* Emmerich and Pettenkofer swallowed cholera cultures the former experiencing a severe attack of cholera and the latter a diarrhoea in which cholera spirilla were present. On the other hand similar experiments have resulted negatively but this might be expected as we know that individuals vary greatly in their susceptibility to infection. In some the organism is destroyed by the gastric juice and others may become cholera carriers without developing detectable symptoms of the disease.

The virulence of the cholera vibrio can be exalted by a series of intraperitoneal inoculations in guinea pigs—especially by direct passage of the peritoneal exudate from one animal to another through a series before culture upon agar. Such a fixed virus the virulence of which cannot be exalted should be employed in the preparation of a vaccine since the writer (1904) showed conclusively that the immunizing power of a strain of *V. cholerae* is in proportion to its virulence.

**Agglutination Test**—By the intravenous inoculation of animals (rabbits or horses) of cholera cultures there may be produced immune sera which are remarkable for their high agglutinating power the titer at times going as high as 1:20,000. In performing agglutination tests for the identification of vibrios isolated from the stools in the diagnosis of cholera one should use a serum of a titer of at least 1:4,000 for its specific vibrio. Such a serum should agglutinate any true cholera vibrio in a 1:500 or 1:1,000 dilution.

**Pfeiffer's Phenomenon.**—The employment of the bacterolytic test *in vivo* is also of great value in identifying the *Vibrio cholerae*. In performing this test a loopful of the suspected agar culture is suspended in 1 cc of normal saline or peptone solution and 1 cc of a 1:1,000 dilution of the cholera immune serum is mixed with it and the mixture introduced into the peritoneal cavity of a guinea pig. Upon removing with a glass capillary pipette a drop of the peritoneal fluid 15 to 20 minutes afterward there is noted in the case of *V. cholerae* an absence of motility and disintegration of the vibrios (*Pfeiffer's phenomenon*). This reaction may also be demonstrated (*in vitro*) in a pipette if fresh immune serum is employed which contains complement. Complement fixation tests using the rice water stools or peptone solution cultures as antigen are of less value than the tests mentioned. The agglutination test is often the most practical for obtaining a specific diagnosis but in some instances it is necessary to employ Pfeiffer's reaction.

Pigeons are almost insusceptible to inoculations of the true cholera vibrio but are readily infected by a closely allied species *V. melnikow* which is not pathogenic for man though pathogenic for guinea pigs.

**Resistance**—The vibrio of cholera has but little resistance to disinfecting agents or to drying. It is also rapidly overgrown by putrefactive bacteria and tends to dis-

appear from sewage-contaminated water in a short time. In water taken from different rivers it has been found to live from 1 to 2 weeks and in the water of a spring for 30 days. Shortt (1939) Director of the King Institute Madras found that *V. cholerae* would survive in water with 1 per cent sodium chloride for 54 days and in 2 per cent sodium chloride for 74 days. He found that the composition of most waters are not suitable for prolonged survival of the organism. In stools it often dies in about 1 or 2 days in summer and in about a week in winter.

**Cholera Toxin**—The absence of *Vibrio cholerae* in the internal organs of fatal cases in which there have been severe general symptoms of the disease during life, points to the production of a powerful toxin produced in the intestines and absorbed by the patient. This toxin is apparently an endotoxin which is set free when the vibrios undergo disintegration especially when lying upon the epithelium of Lieberkühns glands.

Formerly there was some difference of opinion in regard to the exact nature of the cholera toxin and as to whether the organism gave rise to a true soluble toxin similar to that for example of the diphtheria bacillus.

The writer found that if 18 hour agar cultures of the cholera organism are suspended in sterile normal saline solution filtered through a porcelain candle and the filtrate injected into guinea pigs in varying amounts the filtrate possessed very little toxic power. On the other hand if the precipitate remaining on the filter is suspended and injected even though the organisms are killed before injection the guinea pig dies with all the symptoms of cholera intoxication. Hence it seems clear that the toxin is present within the bacteria. If other agar cultures of the organism are suspended in saline and the bacteria carefully killed by heating for a brief period and the bacterial suspensions preserved for 2 or 3 days autolysis occurs aided by the ferments the organism contains. If such suspensions are now filtered through porcelain the filtrate obtained from these killed and digested organisms when injected into animals shows marked toxic properties. On the other hand the filtrates of very young bouillon cultures of the cholera organism are also not toxic for animals and only in filtrates of those cultures in which there are found numbers of dead bacteria which through autolysis have begun to disintegrate is a toxic action observed. The filtrates of old bouillon cultures are much more toxic. Obviously all of this evidence is in favor of the view that the cholera toxin is a constituent of the bacteria or an endotoxin and becomes free only through the disintegration of the cholera vibrios.

In earlier years Melchnikoff Roux and Salimbeni (1896) succeeded in producing death in guinea pigs by introduction into their peritoneal cavities of cholera cultures enclosed in collodion sacs. Banerjee (1932) has again carried out experiments which confirm these facts. Brau and Denier and Kraus also thought that a soluble toxin was produced in alkaline broth cultures of the cholera spirillum. No one however has demonstrated the presence of a true soluble toxin. As pointed out the cholera vibrio possesses its own ferments which are capable of digesting the organisms either in collodion capsules or in alkaline broth cultures autolysis occurs and the toxin is set free. Experiments in immunization also support this view and it has not been found possible by injection of the toxin into animals to produce an antitoxic serum similar to a diphtheritic serum which follows Ehrlich's law of multiples. Experiments carried out by the Scientific Advisory Board of India (1939) are largely confirmatory of these facts.

**Immunizing Properties of Cholera Immune Sera**—Although it has not been possible to secure a serum with high antitoxic power against the cholera endotoxin anti endotoxin sera have been prepared and their action extensively studied. Metchnikoff Roux and Salimbeni of the Pasteur Institute Paris after repeated injections of the toxin into horses and goats found that the serum of these animals would neutralize and protect guinea pigs in amounts of 3 cc against one and one half times the lethal dose of toxine. Brau and Denier of the Pasteur Institute of Saigon immunized guinea pigs and rabbits against the toxin so that they resisted the injections of minimal lethal doses. A horse was repeatedly inoculated intravenously during 6 months with

500 cc of the toxin. From this animal a serum was obtained of which 0.02 cc neutralized 2 minimal lethal doses of the cholera toxin after a contact of 30 minutes *in vitro* prior to its injection into a guinea pig.

MacFadyen in London ground the cholera vibrios at the temperature of liquid air so as to preclude the possibility of chemical change; the organisms then being placed in 10 times their weight of 0.1 per cent liquor potassu. Toxic extracts were obtained from the most virulent cultures which killed guinea pigs in doses of 0.1 to 0.5 cc intraperitoneally and rabbits in the same doses intravenously. Goats were immunized with increasing doses of the endotoxin and a serum was obtained of which 0.002 cc protected guinea pigs against 3-4 lethal doses of the endotoxin.

Kraus prepared a serum for the treatment of cholera by injecting intravenously a horse with cholera toxin at weekly intervals during a period of 10 months until nearly a liter of toxin had been inoculated. He reported that with this serum he was able to save mice which had received one hour before a lethal dose of the toxin or been infected with the cholera spirillum. He found that in guinea pigs if the injection of the serum was delayed for one half hour after the injection of the toxin even large quantities of the antitoxin would not save the animal. Through the intravenous application of large doses of the serum guinea pigs could occasionally be saved after one half hour but after one hour it was of no value.

The writer in Manila prepared an anti-endotoxic serum by repeated inoculation of rabbits intravenously with an extract of the cholera vibrio. Agar cultures of the organisms were suspended in peptone solution and killed by a brief exposure to a temperature of 55 C. They were then allowed to digest at 37 C for 48 hours and the suspensions finally filtered through a Berkefeld candle. By the inoculation of such extracts sera were obtained of which 0.2 cc would neutralize in guinea pigs 4 lethal doses of toxin when mixed immediately before inoculation.

It should be noted that none of these anti-endotoxine sera produced by experienced investigators in different parts of the world has the antitoxic power been sufficient to neutralize in guinea pigs more than 4 lethal doses of the toxin. In the writer's experience if a larger amount of the toxin was injected the animal succumbed even though much larger quantities of the antiendotoxic serum was given. It is also important to emphasize that when cholera immune sera are prepared by repeated inoculations of an animal as a rabbit with killed or living agar cultures of the cholera organism the properties which such a serum exerts in its protection of a susceptible animal as a guinea pig are mainly bactericidal. For example if a guinea pig is inoculated intraperitoneally with 2 mg (platinum loop) of a virulent cholera culture (of which the lethal dose is 0.2 mg) and at the same time or a little later the animal is inoculated intraperitoneally with a small amount (0.5 cc of a 1:100 dilution) of a cholera immune serum obtained as indicated above the cholera vibrios are quickly disintegrated and destroyed by the bacteriolytic action of the serum and the animal survives the infection. If however the inoculation of the serum is not made for 1 or 2 hours after the time of the infection with the living vibrios then even though very large doses of the immune serum are given the animal will die of intoxication. For in this instance even though the great majority of the vibrios may be disintegrated and destroyed by the bactericidal action of the serum the vibrios have already increased so in numbers that when they are lysed sufficient endotoxin is elaborated from the bacterial bodies already present together with that which results from the few surviving organisms that the death of the animal results. Also if one first kills (for example with chloroform) the same virulent cholera organism and inoculates the guinea pig intraperitoneally (1) with the lethal dose of the killed organism (about 8-10 mg) and (2) simultaneously with the immune serum although a union occurs between the bacterial amboceptors of the serum and the corresponding receptors of the vibrios (a fact which is clearly demonstrated by experiment) nevertheless the animal will die for the same reasons expressed above namely that a lethal dose of cholera endotoxin in the bodies of the dead organisms becomes liberated by their disintegration without there being sufficient antitoxin in the serum to neutralize the action of this endotoxin.

From a study of the action of a cholera immune serum in the treatment of the infection in guinea pigs we can perhaps realize why the serum treatment of cholera in man

appear from sewage-contaminated water in a short time. In water taken from different rivers it has been found to live from 1 to 2 weeks and in the water of a spring for 30 days. Shortt (1939) Director of the King Institute Madras found that *V. cholerae* would survive in water with 1 per cent sodium chloride for 54 days and in 2 per cent sodium chloride for 74 days. He found that the composition of most waters are not suitable for prolonged survival of the organism. In stools it often dies in about 1 or 2 days in summer and in about a week in winter.

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or spread and that it had been found possible to forecast epidemics on such data. This does not hold true however for all parts of India. Lal (1939) points out that Rogers' critical level of absolute humidity has not been supported by his studies carried out for Calcutta, Lahore and several other districts in the United Provinces and the Punjab. High relative humidity and high temperatures accompanied by intermittent rains have frequently been found to promote the most favorable atmosphere for the development of outbreaks of the disease not only in parts of India but in China and the Philippine Islands. The Eastern Bureau of the League of Nations health office has recently

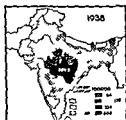
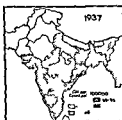
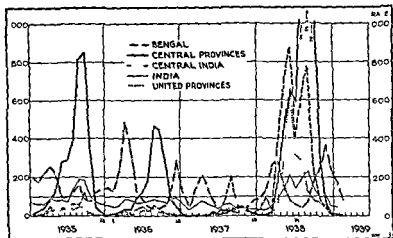


FIG. 149.—Cholera morbidity in India, 1935-1939. (Proportional four-weekly reported annual incidence per 100,000 population) (Epidemiological Service of the League of Nations)

emphasized this fact. However, Lal (1939) at Calcutta, who has investigated meteorological factors (namely temperature, rainfall, relative humidity and number of rainy days) over a number of years, states that in 4 out of 5 homogenous districts the incidence of cholera was independent of the meteorological factors. In the case of the 5th district there was a positive correlation with temperature only.

In lower Bengal and Calcutta, where cholera is present through the year, there has been reported a definite maximal incidence in the dry, hot months of March-June, when the water supply is most deficient and evidently most contaminated, and there is a minimal incidence in the rainy season when the water level is high and water supplies are thoroughly flushed out. Sometimes in certain regions on the Ganges, when epidemic outbreaks have occurred at unusual seasons, the outbreak has been found to

is not more effective. Also in man the small intestine offers a more favorable location for the development and multiplication of the cholera vibrios and one where the serum has not the same opportunities for coming into actual contact with the developing organisms and exerting its bactericidal properties to the same extent as it can in the abdominal cavity of the guinea pig even though the serum may be given in greater quantities to man and be excreted in larger amounts from the intestine. And in fact it has been shown that in cases of cholera with symptoms of marked intoxication the use of these bactericidal sera has not produced any apparent beneficial effect.

It seems probable that in the human body during an attack of cholera anti endotoxin is produced more slowly and in less amount than bactericidal substances and as we have not been able to produce a satisfactory anti toxic serum hence treatment should be particularly directed towards conserving as far as possible the normal processes of the body to withstand the shock of a large amount of endotoxin absorbed within a relatively short period of time. Later the cholera vibrios become greatly diminished in number in the intestine and recovery is likely to occur unless the absorption of endotoxin has already given rise to the production of pathological processes or lesions of a fatal character.

**Epidemiology**—Formerly our attention, as to the methods of transmission of cholera was directed almost exclusively to the water and food supply, with a certain degree of consideration of danger from fomites, especially to that connected with clothing soiled by cholera discharges it having been noted that those who wash such clothing showed a high incidence of infection. Later on the importance of flies in the spread of the disease was strongly insisted upon. However a study of the history of the various epidemics of cholera shows that when infection extends for greater distances it follows the common routes of human intercommunication and is conveyed particularly by man who carries the infecting organism within him. At the present time it is regarded that cholera is most commonly conveyed by cholera carriers or by individuals in the incubation period of the disease or more or less sick with it and it is to the detection and isolation of such persons and cases of cholera that we now chiefly direct our attention in keeping a country free of this dread disease.

Formerly Pettenkofer and Emmerich insisted upon the necessity for certain factors of soil and ground water in the spread of cholera. Emmerich later admitted that the spirilla excreted by carriers can produce cholera but that such transference never gives origin to epidemics. For this to take place he thought that the vibrios excreted by a carrier must come in contact with a soil which has been impregnated with a suitable medium drawn to the surface from the deeper layers of the soil by capillary suction. In such a medium the vibrios flourish and acquire the property of actively producing nitrites from nitrates.

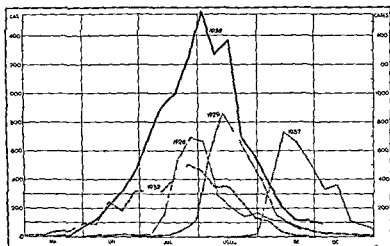
Emmerich considered that the symptoms of cholera are those of nitrite poisoning so that only such organisms as possess this *nitrite forming function* in high degree can produce virulent outbreaks of cholera. This view is regarded today as quite unlikely as an explanation of the pathology of the disease.

Seasonal influences may be of considerable importance in the epidemiology of cholera in regions where the disease is endemic or has more or less temporarily established itself.

Rogers compared outbreaks in various parts of India over a period of 45 years. He found that all but one of 41 epidemics had been preceded by failure of the rains also that an unusually early rise of the absolute humidity favored early recrudescence.

June is hot and the humidity is high. In July and August it is also hot but in addition dry. In September it is hot damp and sultry. After this it becomes cooler. In 1938 the development of an important epidemic was favored in spite of the preventive measures by military operations and the presence in Shanghai of a large number of refugees. However the disease had declined rapidly by October.

Robertson and Polltzer (1939) believe that they have found considerable evidence to support the view that the valley of the Yuan River (which flows into the Yangtze through the Tung tung Lake) is an endemic focus of cholera. In 1931 during the summer following the great flood of the Yangtze a wide spread epidemic of cholera involved most provinces of China took place. Also they believe there is little doubt that the



96 99 I t r n t al S t l m t  
93 I t r n t l t l m t P r e h C o d G t S h g h a i  
937-1918 I t r n t al S t l m t d F n h C o c e a p  
FIG 151—S a o n a l f l u c t u a t i o n n h l e r a m o h d t y a t S h n g h d u r i n g r n t  
p d m y e a r s (w e k l y f i g u r s ) (E p d e m l g c a l I n t e l l g e n e s S r v i c f t b L e g u e  
f N a t i o n s )

wid spread cholera epidemic occurring in the summer and autumn of 1938 first in Hunan and later in adjacent provinces was largely traceable to the Yuan River Valley. Contamination of the river water occurs through the crews of junks who deposit faeces in the river as well as by the transportation of night soil in barges for the use of fertilizing the fields. Cholera was isolated from the river. They believe that cholera has been carried to Shanghai each year from an endemic focus in the mid Yangtze basin probably in the neighborhood of Changteh. They point out that infection in Shanghai has been absent in the periods separating epidemics and that the series of investigations for carriers gave constantly negative results.

**Bacteriophage** — D Herelle has emphasized the importance of bacteriophage in an epidemic of cholera. He and Malone reported only 3 deaths in 41 cases treated with bacteriophage while of 107 cases not so treated 68 died. Note is made of a village outbreak which was stopped by the addition of 30 cc. of bacteriophage to the wells. It is stated that recovery



follow sudden and heavy falls of rain. The epidemic in Central China in 1932 followed the great flood of the Yangtze River.

The League of Nations, in a more recent study of the epidemiology of cholera, explained the considerable differences in the seasonal trend of the disease in the various provinces of India in part by the variations in climate in the volume and flow of the rivers, and in the crops. They found that as a general rule *cholera incidence rises with the temperature and rainfall*. The peak incidence therefore corresponded to summer and monsoon rains in Bihar, Orissa, the United and Central Provinces and Bombay Presidency. The Ganges delta (Bengal) and Madras Presidency were found to be exceptions to this rule.

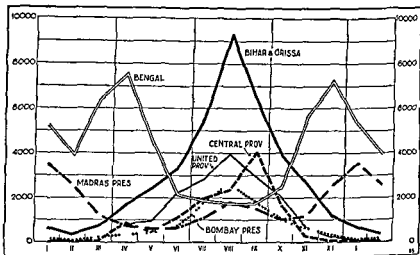


FIG. 150.—Seasonal fluctuations (monthly averages) of cholera mortality in India 1931-1935. (Epidemiological Intelligence Service of the League of Nations.)

The accompanying maps for the years 1935-1939 show how wide the seasonal fluctuations in cholera incidence may be and how important the changes in its geographical distribution in India are. Nearly 94,000 cases of cholera were reported in 1938 in the Central Provinces with a population of about 16,000,000 as a result of the epidemic that began in April 1938.

In China, cholera usually increases in the endemic area of the south coast in spring and spreads in summer to Shanghai and the lower Yangtze valley. However in 1938 as a result of the war and movements of troops and refugees the increase occurred earlier in the year and the disease spread more than usual to inland places. Also the decline in incidence was slower than usual. As has been pointed out cholera usually appears at Shanghai at the beginning of summer and in epidemic form there every 2 or 3 years. It rarely begins before July being well established in August and reaching its peak in August or September dying down in October and disappearing by November. The climate in

the water as taken from the river contained the sewage of Hamburg yet there were only 328 deaths or 2.1 per thousand as against 13.4 per thousand for Hamburg. There were many interesting points in connection with the exemption of certain places in Hamburg of which may be noted the instance of the entire freedom from cholera of a group of houses (Hamburg Hof) with 345 occupants. This was the only section of Hamburg which was supplied with Altona water. As Hamburg and Altona were only separated by the width of a street and hence practically formed a single city the factor of food and contact transmission could easily explain the cases in Altona.

To illustrate the second type of water transmission we may refer to the well known incident of the Broad Street pump. This was about the first definitely proven connection between water and cholera. In 1854 it was noted that cholera was about 10 times as prevalent in Golden Square as in other adjacent parts of London. Various factors such as previous droughts, stagnation of lower strata of the atmosphere, sewerage defects and subsoil drainage were found to be the same in Golden Square as elsewhere. It was noted that the number of cases increased in the neighborhood of the Broad Street well. A large number of cases developed among the employees of a cartridge factory where this well water was used while among the employees of an adjoining brewery which had a well of its own and served out beer to its employees not a single case developed. Very striking was the case of a lady living in Hampstead a section of London which was then free from cholera. This lady had acquired a liking for the water of the Broad Street well and had bottles of it brought out to her regularly. She drank some of the water on the 31st of August, was seized with cholera the next evening and died the following day. A niece who also drank the water likewise died of cholera while a servant contracting the disease recovered.

Macnamara has noted the circumstance of a vessel of water which became contaminated with cholera stools but which at the time it was drunk by 19 persons did not show anything suspicious in odor, color or taste. One person was stricken one day afterward, 2 on the third day and 2 others came down with cholera on the fourth day. It will be noted that only 5 of the 19 were attacked. A similar lack of susceptibility of a certain proportion of people equally exposed has been noted in all cholera outbreaks. It is possible that of those of the 19 who did not contract cholera there were developed a certain number of cholera carriers.

Rain often spreads the infection from cesspools into the water supply and into surface wells in rural districts in the tropics. Also the individual water supply may become infected through water carts or through water barrels. In the Philippines or in India the wells not infrequently became infected. In the Philippines they were disinfected by chlorination or other artesian wells were bored.

In countries which lie adjacent to endemic centers of infection the disease also may spread considerable distances by an infected water supply. Thus in India the infection has been carried by the River Cauvery for approximately 18 miles to the Madras Presidency. The infection was also said to be carried from Lake Fife (the source of the water supply) which became infected through water pipes for a distance of at least 1 miles. And in Mesopotamia cholera infection has apparently travelled long distances down the Tigris River. Robertson and Pollitzer have also pointed out that in the cholera epidemic in Central China in 1938 the infection was transmitted especially by the Yuan River and that the river water contaminated with cholera vibrios formed the most important vehicle of infection.

**Food Transmission.**—Cholera infection is acquired by way of the mouth and alimentary canal especially through drinking water and by ingestion of food and some

or death is intimately associated with the presence of bacteriophage or the lack of it and that it is the appearance of bacteriophage which destroys the cholera organism and thus cuts short an epidemic

Asheshov added mixtures of 3 bacteriophages to the water of wells in the villages and towns in one district in Bihar and reported there was a marked drop in the incidence of cholera. However this was not confirmed by Russell although Morrison has also claimed good results in Assam by adding phage to water supplies

**Water Transmission**—There are 2 types of outbreaks of cholera according as the general water supply is contaminated or when such contamination is localized to certain wells, cisterns or other nongeneral supplies. In the former the onset is explosive and cases occur almost simultaneously and with equal distribution in all parts of the city to disappear with almost equal suddenness. In the latter mode of infection cases will appear from day to day and often peculiarly localized to certain definite districts of a city or to certain definite users of a particular water supply

As an example of the first type of outbreak the Hamburg epidemic of 1892 is most instructive. During a period of only about 2 months

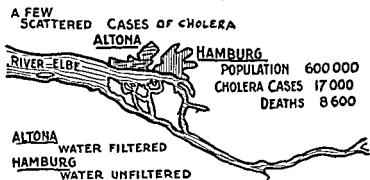


FIG. 152—An instructive contrast between Altona and Hamburg before the latter filtered its water having learnt its lesson from a sharp outbreak of cholera. (After G. E. Armstrong.)

cholera attacked about 17 000 persons causing 8605 deaths in a city with a population of 600 000. This outbreak was attributed to the washing of clothes in the water of the Elbe River by Russian immigrants. These immigrants had come from cholera infected districts and among them it has been said there undoubtedly were cholera carriers. However it has also been suggested that it is much more likely that the water supply was contaminated by the direct inoculation of it with bacteriological cultures of the cholera vibrio by some laboratory worker in Hamburg. A number of German bacteriologists were especially studying the disease at that time.

The water supply of Hamburg was taken directly from the river. The adjoining city of Altona with a population of 140 000 was further down the river but filtered its water by a slow sand process. Although

the consumption particularly of raw tunny fish and boiled or broiled halibut or mackerel. Such cases were largely confined to the coastal cities and villages.

*Transmission by carriers* or by individuals in the incubation period or more or less sick with the disease or convalescent from it are now generally recognized as one of the most important factors in the spread of cholera. The individual who is excreting vibrios while in apparent health is sometimes far more dangerous than the one excreting such organisms in the rice water stools of a well recognized case of the disease as he may go about undetected. Dunbar was the first to draw attention to the presence of virulent cholera spirilla in the faeces of apparently healthy persons during the Hamburg epidemic of 1892.

Since that time these observations have been generally confirmed. In some instances as many as 20 per cent of those who have been in immediate contact with a cholera patient have become carriers, some showing symptoms of cholera but a larger proportion excreting cholera spirilla while continuing in health. While cholera prevailed in Manila McLaughlin reported from 6 to 7 per cent of vibrio carriers among healthy persons living in the infected districts.

Pottevin reported that of 1300 pilgrims examined 17 per thousand carried cholera vibrios. The carriers were especially common among the dysenteric patients. During the Naples epidemic of '91 it was reported that on the average 10 per cent of healthy people in contact with cholera cases became carriers. It was estimated that 90 per cent of the cases in this epidemic were infected by sick or healthy carriers.

Sergeant reported the case of a healthy carrier who continued to excrete cholera vibrios for 2 months and during this time was in contact with 8 persons 7 of whom became infected and 4 died. In Manila it was found that a number of children reported as dying of meningitis or infantile beriberi were cholera cases.

The vibrios are rarely excreted in the faeces of the cholera patients longer than 7 to 10 days, exceptionally for 14 days though they frequently disappear in 3 or 4 days.

With healthy cholera carriers the period of the continuance of vibrio excretion is equally short but cases have been reported where periods of from 3 weeks to 2 months have been noted. It is usually stated that 97 per cent of carriers become vibrio free within a month.

Greig has found injection of the bile of the gall bladder or ducts in 80 cases in 271 cholera autopsies. When living organisms are injected into the ear vein of a rabbit they sometimes pass into the bile. An examination of the epithelial layers of the gallbladder of such a rabbit may show destruction of the cells and the presence of vibrios in the underlying tissues. While cholera organisms are soon crowded out by intestinal bacteria thus explaining the short period during which cholera vibrios are excreted by convalescents this may not be true when the cholera vibrio gets into the bile ducts or gall bladder. Greig found one cholera convalescent excreting cholera vibrios 44 days after the attack. Of 27 persons who had been in contact with cholera patients 6 were excreting cholera vibrios although apparently well. A very important matter is that persons who fail to show cholera vibrios may begin to excrete vibrios after the administration of a purgative or following some intestinal disorder. In fact it has been reported that purgatives may set up an attack of cholera in a cholera carrier.

times by contamination of the hands with infected material. Food contaminated by dejecta from cholera patients or carriers is dangerous in proportion to its condition of moisture. Drying and the development of inimical organisms may be important factors in destroying the cholera vibrio. Temperature and sunshine are operative in assisting the drying process.

During an epidemic uncooked fruits, vegetables and salads may become infected.

Lettuce and celery are particularly dangerous because of the favorable condition of moisture in their folds and imbrications. Furthermore these vegetables are eaten uncooked and may have been fertilized with night soil (human excrement) which material if containing cholera dejecta would infect the plants. Milk is an excellent culture medium for cholera vibrios but upon becoming acid may sterilize itself of these vibrios. In sterilized milk however the cholera vibrio may live for extended period as long as 60 days and even when such milk is contaminated by faecal material containing other organisms besides the cholera vibrio the vibrios live much longer than they do in raw milk.

Milk is liable to be contaminated by flies which have been in contact with cholera stools. In cholera hospitals ice chests containing ice and foodstuffs have sometimes been infected by the hands of attendants and nurses. Infected clothing and the washing of such clothing from cholera patients in streams and the subsequent drinking of the water has not infrequently given rise to the disease. Water that has been boiled and food that has been cooked should subsequently be scrupulously protected from flies or other contaminating agents.

Flies may play an important part in spreading the infection mechanically by settling on articles of food shortly after having been in contact with infected material especially the faeces of cholera cases. Transmission by flies in this way seems to have been important in an outbreak in Shanghai in the fall of 1937. Soparkar (1939) reports that in his experiments the vibrios ingested by flies are either rapidly excreted or destroyed in the gut and there is apparently a vibriocidal action of extracts of the crop and intestine. However he did isolate cholera vibrios from flies in a number of instances from 2-12 hours after the interval of feeding of the flies on infected material.

Pasricha (1939) found the vibrios he isolated from flies and cockroaches were agglutinable by Inaba O serum. On the other hand Taylor (1939) found a number of strains isolated from cockroaches and water supplies all agglutinable with Inaba O serum. In any case one cannot disregard the mechanical transmission of the infection by flies. Robertson and Pollitzer (1939) believe that flies played a considerable part in cholera transmission during the summer months in Central China. They point out that the end of the watermelon season in mid August was coincident with the decline in cholera. The watermelon as such was quite innocuous when the fruit was consumed freshly cut. The danger lies in the exposure of cut slices to flies and in the watering of these to keep them fresh with a filthy rag dipped in polluted water.

Uncooked shell fish may be peculiarly dangerous in cholera outbreaks. In India sun dried fish which are frequently covered with flies during the curing process are a factor in the spread of cholera. In Japan and Korea where severe epidemics not unfrequently arise the Japanese consume food in an uncooked state perhaps more largely than any other people. Raw fish is a favorite article of diet and the cholera vibrio is capable of living in the intestines of fish taken from cholera infected water and such fish are difficult to disinfect. Even light broiling or boiling may not destroy the cholera vibrio in them. The epidemic in Japan in 1922 apparently originated through

matter Of particular importance is the fact that so many sick people make pilgrimages these being peculiarly liable to act as carriers Excesses in eating often of badly prepared or decomposing food following periods of religious fasts predispose the natives of India to cholera

Lowered resistance as from disease or from gastric disorder increases the susceptibility to cholera Errors in diet and in particular the effects of alcoholic excesses markedly predispose to infection In India cholera often accounts for about 1-2.5 deaths per 1000 of population in the different provinces

### PATHOLOGY

It has been shown that the cholera vibrio apparently does not produce a true soluble toxin the toxine being an endotoxine The organism rarely penetrates more deeply than just under the epithelial layer of the glands of Lieberkühn During the stage of evacuation as a result of the outpouring of the fluid into the lumen of the gut a concentration of the blood plasma occurs and there is an increase in the red cells (7 000 000 per cu mm) and a leucocytosis of from 12 000 to 50 000 The specific gravity of the blood is greatly raised 1.073-1.078 and the alkalinity diminished The blood pressure is markedly lowered 60 mm in very severe cases and 75 mm in less severe ones

The lower portion of the small intestine appears to suffer particularly from the local action of the endotoxin of cholera Early and marked postmortem rigidity is a striking characteristic of the cholera cadaver Muscular contractions causing odd positions of the limbs have at times given a basis for the idea that the victim had been buried alive Besides marked rigor mortis the emaciation leaden hue of skin and shrivelled hands are noteworthy

In opening up the body there is a striking dryness of all the structures The dry and dark red muscles stand out prominently The lungs are dry and shrunken The right heart may be full of a dark jelly like viscid blood The leading changes are found in the abdomen The omentum is dry sticky and shrivelled looking The intestines have a ground glass appearance with a lilac pink color of the small intestines which is in contrast with the normal color of the large intestines There is congestion of the affected intestinal mucosa and the lumen is filled with an alkaline material resembling rice-water If death has occurred late in the disease the contents of the bowel may have a rather brownish appearance and a foul odor When death occurs during the stage of reaction the stools are moister and less congestion of the venous system is present Nevertheless in some instances the lungs may be congested and oedematous There is usually a parenchymatous nephritis and on section the medullary portion of the kidney much congested Chatterjee (1941) has made a histological study of the kidneys in cholera Acute inflammatory changes were absent as a rule though acute congestion of the medulla and glomerular capillaries might be seen The changes observed in non uraemic kidneys were much less marked than in uraemic ones

The recognition of cholera carriers has been made by the isolation of vibrios from the stools which agglutinate in proper dilutions with a known cholera immune serum. However it must be borne in mind that there are a number of other vibrios which imitate *Vibrio cholerae* precisely in staining and cultural characteristics. Such vibrios are found in the intestinal tract of man and also as free living forms especially in river and well waters. However in a typical case of cholera usually the cholera vibrio can be distinguished from such organisms by its serological reactions with a cholera immune serum (the agglutination test and Pfeiffer's phenomenon).

Nevertheless in endemic centers during interepidemic periods and in the early and late stages of cholera epidemics nonagglutinable vibrios are frequently encountered in the stools of man. There has been an old theory that under unexplained conditions nonagglutinable vibrios may develop pathogenicity and produce outbreaks of cholera. Brahmachari in a series of such examinations near Calcutta found only 0.4 per cent with agglutinating vibrios while 13.7 per cent had non agglutinating vibrios in their stools during the months from June to November. In November and December agglutinating vibrios were present in 13.7 per cent of stools. He believes that non agglutinating vibrios are but agglutinating ones transformed by environment. He found that a number of nonagglutinating strains after 6 months cultivation became agglutinable. Tomb and Maitra (1927) have also considered that agglutinating vibrios are merely different phases of nonagglutinating ones. However other bacteriologists feel that this has not been conclusively demonstrated.

In the investigations at the Medical Research Institute at Shillong (1939) it was not found possible to transform agglutinable to nonagglutinable vibrios. On the other hand it was noted that in certain undoubted cases of clinical cholera it seemed impossible to find agglutinable vibrios and that the presence or absence of phage for the infecting vibrios in nature did not appear to influence their agglutinability.

Pasricha and his associates (1931) believe that there is some bacteriophagic and serological relationship between the cholera vibrio and the cholera like vibrio. They suggest that some of the nonagglutinating vibrios in man where cholera exists may be mutation forms of the true cholera vibrio and may under certain conditions when placed in a suitable environment play an important part in the etiology in a new outbreak of cholera. There is still much discussion as to the reliability of the agglutination test in detecting *Vibrio cholerae* under all conditions.

**Influence of Pilgrimages**—The spread of cholera has frequently been intimately connected with the great religious festivals and pilgrimages of Oriental people. Not only do those of India keep up the dissemination of the disease there but pilgrims going from the delta of the Ganges to Arabia and Mecca carry the infection and transmit it to their fellow pilgrims from Egypt and Algiers. Hardwar which is a great pilgrim center, appears to be of great importance as a disseminating area of the disease the majority of pilgrims traveling there from the Punjab. Lal (1937) has emphasized the importance of the periodical festivals with reference to the epidemiology of cholera. One of these fairs is held on the grounds at the confluence of the Jumna and Ganges and lasts a month. At the 1936 fair there were about 50,000 temporary residents and the bathers on the most important day of the festival numbered a million and a half. The latrines are frequently situated near the numerous wells. Greig examined a number of cholera convalescents who were about to return to their homes in India and found that 30 per cent of these pilgrims excreted cholera vibrios in their stools. The epidemic in India in 1939-1940 reported by Verghese occurred in connection with the pilgrimage in the vicinity of the Jaganath Temple where 75,000 people congregated.

The intimate commercial relations between Europe and Egypt and Algiers make the introduction of the disease into European ports an easy

ciency in water and salts the cells and albumin are in excess and hence the blood has a high specific gravity. The severe purging and vomiting having brought about a concentration of the blood the red corpuscles are found to be increased and there is a corresponding rise in the percentage of haemoglobin the amount of oxygen in the red blood corpuscles becomes diminished and the blood becomes very dark in color. Usually there is a leucocytosis. Urea has been found in the blood in fatal cases in the algid stage but it has not been possible to detect the cholera toxin in the blood.

In the stage of collapse in cholera there is in many cases a marked loss of water from the blood accompanied by a corresponding loss of salts particularly chlorides. This loss in water is particularly high in persons who have died of the disease. In the later stages the blood may show an almost normal amount and proportion of water but the salts are not always replaced in normal amounts and proportion. Therefore the blood at this stage may have a diminished salt content and be hypotonic and its alkalinity reduced. These changes may be of importance in reference to the treatment of the disease.

### SYMPTOMATOLOGY

**Typical Cases**—In a typical attack of cholera it is frequently possible to distinguish certain clinical stages of the disease in which the symptoms vary. In some instances an incubative stage can be recognized followed by one of evacuation in which purging vomiting and muscular cramps are the most prominent symptoms. This condition in severe cases is usually followed by an algid stage or one of collapse and if death does not occur a period of reaction follows in which the temperature rises and if no complications supervene the patient usually recovers.

The *period of incubation* is usually from 1-5 days more commonly not over 3. The symptoms which may occur are diarrhoea a feeling of weight or oppression in the stomach and occasionally nausea and vomiting. In some cases a premonitory diarrhoea appears to favor the development of the cholera vibrio in the intestine.

**The Stage of Evacuation**—However in many cases premonitory symptoms are not observed and when the physician sees the patient this stage may have passed. The onset of a typical attack as observed during epidemics is frequently abrupt with purging vomiting muscular cramps and exhaustion.

These symptoms dominate the *stage of evacuation* which usually lasts from 2-12 hours the time depending probably on the virulence of the infecting cholera vibrio and the susceptibility of the individual. The purging is usually frequent copious and watery but there is neither colic nor tenesmus. In fact the stools are often voided with a sense of relief as when an enema is gotten rid of. A striking feature however of the movements is the sensation of prostration which accompanies and

Recently the pathological conditions which result in surgical shock have been compared to those which occur in Asiatic cholera. While there are of course certain similarities it should be kept in mind that in Asiatic cholera the pathological conditions are first produced through the toxins of the cholera spirillum and one should not ignore this fact. If a guinea pig is injected intraperitoneally with 2 mg. of a virulent culture of the cholera spirillum the organs multiply rapidly and death occurs in collapse within 24 hours after the inoculation but there is no loss of fluid from the intestinal tract and no dehydration. The animal dies from the effects of the cholera toxin acting on the organs.



The following features are especially characteristic of cholera at autopsy

(1) Early and marked rigor mortis (2) the shrunken pinched face with cyanosis about the eyes and shrunken eye balls (3) shrunken washerwoman's fingers and cyanotic finger nails (4) dry subcutaneous tissue and muscles (5) dry and sticky peritoneum with ground glass appearance of serosa of the intestine and with pink or rosy appearance of the serosa of the ileum (6) contracted and empty urinary bladder (7) shrunken dry spleen (8) rice water like intestinal contents (9) some prominence of lymphoid follicles in the ileum

Greig drew attention to the frequency of the involvement of the gall bladder. He also noted the presence of small areas of consolidation in the lungs of those developing pneumonia during the early days of convalescence. In the exudates of such areas, cholera vibrios could be seen thus showing their penetration to the lung. Although rare instances of recovery of the cholera spirillum from the blood have been reported Greig was unable to accomplish this in any instance. The writer in many necropsies in the Philippine Islands never found the cholera vibrio in routine cultures made from the blood.

Recent studies of the blood have been made by DeMonte and Gupta (1938), who made blood cultures from 26 patients and in 20 within 9 hours of the onset of the symptoms. In no case was the cholera vibrio isolated.

In Greig's opinion the vibrios may travel by way of the lymphatic system but this has not been demonstrated. In 8 cases out of 55, he recovered the vibrios from the urine. However other investigators have failed to find *Vibrio cholerae* in the urine and doubt its occurrence in the bladder. Chatterjee (1938) in a recent Indian epidemic also was unable to isolate vibrios from the urine.

**Clinical Pathology**—In the stage of evacuation following the premonitory one often within a few hours several liters of saline fluid are passed from the intestine or expelled from the stomach by vomiting. This results in more or less dehydration of the tissues and blood a fall in the blood pressure and reduction of the surface temperature with marked weakening of the pulse, palor of the skin, muscular cramps and sometimes complete suppression of urine. These symptoms result especially from the osmotic processes which occur during the course of the disease. In relation to the loss of fluid from the body there is (1) a transudate of fluid from the vessels into the intestinal canal (2) an exudation from the corpuscles into the surrounding fluid and (3) passage of fluid from the tissues into the vessels. In this way the blood becomes profoundly altered both physically and chemically.

The change in the constituents of the blood has been shown by Schmidt and Aron in the investigations instigated by the writer in the Philippines to occur in the following order—the water transudes before the solids of the serum, the inorganic before the organic solids, the chlorids before the phosphates, and the salts of soda before the salts of potash. Shorten also found a retention of phosphates in the blood. The alkalinity of the blood becomes gradually diminished and the percentage of chlorid in the serum in some of the most severe cases is greatly reduced. In some cases the glycogen disappears from the blood and a mere trace may be left in the liver. Owing to the defi-

ciency in water and salts the cells and albumin are in excess and hence the blood has a high specific gravity. The severe purging and vomiting having brought about a concentration of the blood the red corpuscles are found to be increased and there is a corresponding rise in the percentage of haemoglobin. The amount of oxygen in the red blood corpuscles becomes diminished and the blood becomes very dark in color. Usually there is a leucocytosis. Urea has been found in the blood in fatal cases in the algid stage but it has not been possible to detect the cholera toxin in the blood.

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follows them. At first the movements are diarrhoeal or faecal but they soon assume a rice water appearance, and are frequent and copious. This designation is an appropriate one as the flocculi consisting of intestinal epithelial cells in the watery, slightly opaque, albuminous fluid much resembles rice water. The odor is also slightly albuminous. A short time after the purging begins vomiting usually occurs. At first everything the stomach contains is ejected, later the vomitus usually assumes much the same rice water like character as the stools and at times gushes from the patient's mouth causing much distress. It may become blood tinged. Any nourishment given by the mouth is apt to excite vomiting. Retching which is very exhausting, and hiccough are also frequent symptoms. Through loss of fluid from the body, the red cell count may become increased from 1-2 millions per cubic millimeter. Early in this stage agonizing cramps frequently appear in the muscles of the calves of the legs and feet thighs and of the arms. Frequently the patients suffer greatly from this condition and sometimes cry out for relief. The muscles of the abdomen and back may also be involved as may at times the muscles of almost any part of the body.

The appearance of the patient may change rapidly the skin becoming lax and wrinkled. Along with the excessive loss of fluid the tissues especially of the face become shrunken, the eyeballs with their congested conjunctivae sink back in the sockets and the nose becomes pinched the cheek bones standing out prominently. This with the cyanosis about the eyes and lips the half closed pupils, and apathetic expression constitutes what is known as the 'cholera facies'. Cyanosis may gradually become evident about the fingers and toes as well as about the eyes and mouth. The hands often have the appearance of having been held in water for a long time the so called washer woman's fingers. The heart impulse and heart sounds which have greatly increased in frequency become gradually weaker. The pulse becomes more and more feeble and there is a steady diminution in the secretion of urine. Unquenchable thirst is a very common symptom. Faintness and palpitation of the heart are frequently complained of. The temperature of the surface of the skin is often subnormal while in the rectum the temperature may reach 101°F or 102° and in very severe cases as high as 104°F. Consciousness is usually retained. An increased dusky skin of the skin which is cold and clammy to the touch may denote the onset of the algid stage.

**The Algid Stage**—In severe cases the disease passes to the algid stage but perhaps more appropriately termed the stage of collapse. This may last for only several hours or be prolonged for several days. The purging and vomiting often cease but alarming exhaustion follows. Colorless movements may be still occasionally passed involuntarily, and retching may persist. The muscular cramps may also still continue.

The most serious symptom of the algid stage is almost complete cessation of the circulation in the severe cases. The pulse which may have increased to 120-160 grows gradually weaker becomes irregular

and finally can no longer be felt at the wrist. Sometimes pulsation is imperceptible even in the larger arteries. If a vein is incised only a few drops of dark tarry blood may exude while if a small artery is cut the blood also may not flow. The blood pressure may fall to 60 millimeters. The heart sounds become weak and irregular the first often prolonged. Friction sounds may be heard in the pericardium and sometimes in the pleura as a result of lack of moisture. The secretion of urine diminishes rapidly both on account of the low blood pressure and of the great loss of fluid through the bowel discharges. In the most severe cases the pressure may fall in the renal arteries to below 40 milligrams of Hg which pressure is necessary for the secretion of urine. Notwithstanding the intensive exhaustion of the patient and his cadaveric appearance the mental faculties may be fairly preserved.

The temperature of the sodden inelastic clammy skin is depressed and may be as low as 95-94 F while the rectal temperature may be approximately normal or be elevated.

The poor circulation and poor aeration of the blood may stimulate the respiratory centers and the respirations become shallow and rapid. The voice becomes husky and finally so feeble that the patient can only whisper and the breath feels cold.

The sodden shriveled hands resembling those of a washerwoman become very characteristic. The thirst usually becomes intense. The patient frequently falls into a listless motionless state in which however the apathy is more apparent than real. The cholera facies often becomes even more accentuated in which the features are pinched the skin drawn the eye balls sunken and surrounded by dark bluish areola. The pupils are half closed and the expression apathetic. Usually in this stage there also is no delirium though the intelligence may be cloudy. Later a comatose or semicomatose condition may result. Death may take place in this stage from respiratory failure asthenia or coma. Sometimes death occurs a few hours after the onset of the symptoms in other cases not until the second day. In severe cases collapse and uraemia are the 2 most frequent causes of death. However more than half the patients usually survive the algid or collapse stage. Those who survive usually pass on about the third day into the stage of reaction.

**Reaction Collapse and Uraemia**—In the *stage of reaction* the acute symptoms disappear the stools become of greater consistency and may contain traces of bile. The skin becomes warm to the touch and the temperature by mouth may register several degrees above normal. The pulse may be felt at the wrist the cyanosis disappears and the urinary secretion usually increases. Recovery may take place within a week. However in unfavorable cases the urinary secretion does not return to normal and only a small amount of albuminous urine is passed. In such cases the pulse may become full and bounding and the systolic pressure increased to 150-175 millimeters. A typhoid state may ensue with accelerated respiration dry brown tongue and muttering delirium. A condition of acidosis may develop with the carbon dioxide content of the

blood greatly diminished Erythematous skin rashes may appear If the anuria persists, the prognosis is grave and uraemia convulsions coma and death may occur Uraemia, often associated with acidosis is the most serious complication in the late stages of cholera

**Types of Cholera** —During an epidemic, the different cases of cholera vary greatly in their severity While at the height of the epidemic the majority of the cases are severe especially in the early or late periods of the epidemic, many cases occur in which the symptoms are considerably milder Some of these may be *ambulatory* in character the symptoms consisting generally of slight gastro intestinal disturbance accompanied by diarrhoea The stools while liquid may never become colorless or assume a rice water like appearance In such cases urinary disturbances are usually not observed and the attacks usually subside in a few days However, the cholera spirillum has frequently been isolated from such ambulatory cases and as they may constitute sources of infection for others they should be regarded as 'cholera carriers' Other patients may have more characteristic rice water like stools which persist for 48 hours but the cases are not characterized by suppression of urine or muscular cramps Stitt and others have referred to this group as cases of '*cholerae*' or of choleraic diarrhoea However some of these patients may develop acute and more serious symptoms of cholera In a small percentage of the cholera cases during epidemics a condition known as *cholera succa* has been reported Stitt points out that this type of disease is more apt to be seen in old or debilitated people In this form death occurs from collapse apparently from the rapid absorption of the cholera toxin before symptoms of vomiting or diarrhoea appear In such cases at autopsy the small intestine is usually distended with rice water material Apparently this form of cholera is rare

### SYMPTOMS IN DETAIL

**General Appearance** —A typical case of cholera, with its cyanosed drawn pinched face cold clammy skin and the eyes deeply sunken in the orbits, produces a picture rarely seen in other pathological conditions The appearance of 'washerwoman's hands' is especially characteristic

**Alimentary System** —In the beginning of an attack of cholera the stools may be bile stained but in a typical case with sudden onset the cholera evacuations consist of a colorless or grayish white opalescent fluid containing flakes which resemble particles of boiled rice The typical cholera stool resembles very closely moderately thick rice water and may contain as much as one half per cent of sodium chloride The watery dejections are frequently very copious and violent Sometimes approximately a liter of fluid may be ejected within a few minutes or several liters within a few hours As the disease progresses the dejections usually decrease in frequency and amount and as convalescence begins the stools assume a light yellow or a faint greenish tinge Microscopical examinations of the rice water like evacuations reveals that the floccules or rice like particles consist of particles of mucus and degenerating intestinal epithelial cells Sometimes very few cells are recognizable these having undergone more or less granular degeneration Red blood corpuscles in small numbers may also be visible but polymorphonuclear leucocytes and endothelial cells are usually scanty If a rice like particle is examined on a hanging drop slide under the microscope vibrios with

lively motility are usually seen. If the preparation after drying is hardened in methyl alcohol and stained in dilute carbol fuchsin solution, so an almost pure film of the cholera vibrio is often found. Even though other intestinal bacteria are present, the appearance of the cholera vibrios is striking, not only on account of their comma or spiral shape but because they usually assume a lighter tint than the normal intestinal bacteria. However, in the later stages of the disease in some mild cases of cholera or in cholera carriers, it may be impracticable to detect by microscopical examination alone the presence of vibrios in the dejecta. In some mild cases, while a microscopical examination of the stool may not reveal the cholera vibrio, nevertheless this organism may be isolated by cultural methods. Sometimes a cherry red color (indicating the presence of indol) may be obtained if a small amount of an alcoholic solution of para-dimethyl amido benzaldehyde and an aqueous solution of potassium persulphate is added to the rice water like stool.

Vomiting usually appears either a little later or at about the same time with the purging. It is frequently persistent. As soon as the stomach has been thoroughly emptied of food, the vomitus becomes watery in character and later assumes a more or less rice water like appearance. It may take on a bile tinge or be reddish and contain a small amount of blood. The vomiting is often copious and as much as a quart may be ejected in a few minutes. Repeated vomiting of large amounts is a very striking feature of the disease. It is frequently very exhausting to the patient and may contribute markedly to the collapsed condition, occasionally being followed by rapid heart failure. Great thirst is usually present, but if water is given the stomach is often unable to retain it and it is often immediately ejected. However, when very small amounts of rice or of fluids are given a small amount may be retained.

Hiccough likewise is a frequent and difficult symptom to treat. It often persists after vomiting has ceased and also during the collapse stage.

**Circulatory System.**—The pulse is rapid and feeble in the stage of evacuation but often becomes imperceptible in the algid or collapse stage. The circulation is seriously interfered with so that only a few drops of black tarry blood which does not coagulate readily will flow from the wound made in a vein when one gives an intravenous injection. Especially as the result of pathological osmotic processes referred to under pathology, the blood becomes concentrated and has a high specific gravity varying between 1.060 and 1.078. The systolic pressure falls greatly, even to 50–60 mm. of mercury in severe or 75 mm. in less serious cases. The red cell count is increased to 7 or 8 million red cells per cmm. and the leucocyte count reaches 15,000 to 30,000, rarely 50,000.

In the stage of collapse, loss of water from the blood is accompanied by a corresponding loss of salts, particularly chlorides, and this loss is constantly high in the blood of persons who have died of the disease. In the later stages of the disease, the blood again shows an almost normal content of water, but the salts are not replaced in their normal amounts and proportion. Therefore the blood at this stage has a diminished salt content and is hypotonic and its alkalinity is usually reduced. In the later stages of the disease, Shaker and Sellards found that sometimes the CO<sub>2</sub> content of the blood may be greatly reduced.

Rogers and Sherten also showed that a greatly reduced alkalinity of the blood is a common feature of severe cholera; the alkalinity of the blood not rarely being reduced from a normal of about N/20 to as low as N/60 to N/8, and in cases terminating in fatal suppression of urine to N/100 and even lower. Such extreme cases of acidosis are almost fatal.

Bancroft (1941) emphasizes that the excessive vomiting and diarrhea both lead to great loss of chlorides. In a series of cases in which measurements were made, it was found that a loss of 9.7 gm. chloride in 24 hours occurred through vomiting and of 34.6 gm. by the bowel. These cases were receiving at the same time a fair quantity—about 25 gm. of chloride daily by intravenous and subcutaneous injection. The result was a marked hypochloremia with a great alteration in the distribution of electrolytes and in the acid base balance. He points out that it is the hypochloreaemia that is of more importance in the production of a coma than the concentration of the blood. If the loss of chlorides remains unrestricted, the invariable result is dehydration, retention of nitrogenous waste products and renal failure.

**Temperature Record.**—The temperature of the skin is lowered in the stages of evacuation and collapse from the normal while that of the rectum may be normal or even elevated. There sometimes may be a difference of 10 F. between rectal and surface temperature. In the stage of reaction a rise of temperature usually occurs in severe cases to 100–102 F. In grave cases it may continue to rise to higher fever points 103–104 F. and this so called hyperthermic type is very fatal. If uraemia occurs the case may assume a typhoid form with a temperature continuously high of 102–103 F.

**Urinary System.**—The urine usually has a high specific gravity. It almost always contains albumin and casts and is scanty in amount. Suppression not infrequently occurs. In severe cases albumin is present in considerable amounts during the first 2 or 3 days of the attack. After this time in those who recover the albumin becomes gradually decreased. However if uraemia develops the amount of albumin generally continues high. The urea is much reduced during the first 2 days of the attack and generally increased in amount from the third day in cases which recover. However in those who develop uraemia both the urea and total solids may remain decreased as well as the quantity of urine passed.

The urinary system is greatly disturbed in cholera. As soon as the blood pressure falls sufficiently the amount of urine becomes diminished or anuria results. That the failure of the kidneys to excrete urine is due in part to the lowered blood pressure is sometimes demonstrated by the fact that following the intravenous injection of a saline solution for treatment the urinary secretion returns. However in those cases in which the parenchymatous cells of the kidney have already been seriously injured perhaps by the cholera toxin as well as by the circulatory changes the secretion of urine may not be restored. The continued failure of the renal function leads quickly to uraemia which is usually the most serious complication of cholera. Sellards (1936) pointed out that uraemia proves fatal in about 15 per cent of all patients who contract cholera. After the period of reaction the secretion of urine may return and then recovery usually takes place. However in cases in which uraemia develops the bladder often remains empty or only a few ounces of urine are passed and the symptoms of toxæmia rapidly develop. In such cases the pulse remains above normal and tends to increase. The blood pressure remains high and the respirations become more rapid and deeper. If the secretion of urine is not restored the respirations become more and more labored the intellect becomes cloudy and coma or convulsions frequently ending in death supervene. Even after relatively enormous injections of bicarbonate of soda the urine may remain sharply acid. In some instances after the injection of 90 grams it did not become alkaline whereas in normal individuals from 3–5 grams is sufficient to change the reaction of the urine from acid to alkaline. Further studies demonstrated that this tolerance to bicarbonate in cholera is due essentially to acidosis or to a deficit of the body in fixed bases. This acidosis makes its appearance usually early in the stage of reaction and reaches its maximum in cases showing the most profound evidence of uremia.

**Other Prominent Symptoms**—Muscular cramps particularly of the extremities and abdomen are often of agonizing character. They appear during the stage of evacuation and are apt to be severe in the stage of collapse and to occur when the circulation is profoundly disturbed. They are apparently due particularly to the poor circulation of the viscid venous blood deficient in oxygen as when the circulation is restored by intravenous saline injections the cramps usually disappear at least temporarily.

In pregnant women cramps of the abdomen and uterine muscles may aid in producing abortion which usually occurs in cholera. In the extremities the muscles during the cramps are hard to the touch and stand out rigidly sometimes having a knotted feeling from the violence of the contractions.

A striking feature in regard to the *nervous system* is that the mind is clear during the evacuation and algid stages even when the patient seems profoundly apathetic.

#### COMPLICATIONS AND SEQUELAE

Uraemia is the most frequent and fatal complication and in different epidemics it has caused from 15–25 per cent of the deaths. Its association

with acidosis has already been considered. Although the disturbances of the kidney and urinary secretion are marked in the acute stages. Bright's disease is an unusual sequel of cholera and in the majority of the cases which recover the urine soon becomes normal.

The cholera typhoid state which occurs in about 25 per cent of the cases usually becomes manifest after the stage of collapse. There is a rising temperature, accelerated respiration and sometimes a dicrotic pulse. The face is frequently flushed, the tongue dry and brown. There is often muttering delirium. The typhoid state has been regarded by some as a result of the cholera intoxication. However it seems probable that the other bacteria in the intestine which may cause secondary infection through the damage to the intestinal epithelium may exert an influence and give rise to symptoms.

In many of the cases of typhoid form uraemia occurs. In these the urine is sometimes delayed for many hours or even days. This is usually a serious symptom and in the majority with prolonged suppression cerebral symptoms make their appearance such as stupor, restlessness, muttering delirium and twitching of the muscles. The pulse is usually slow and the pupil often contracted and fatal coma may follow. However if the urinary secretion is reestablished in treatment the alarming symptoms may subside and recovery take place.

Among other complications should be mentioned persistent hiccough which is not uncommon. Cholecystitis may occur and be accompanied by severe pain in the right hypochondriac region. Jaundice is rare but is regarded as a dangerous complication. Parotitis occurs in about 1 per cent. In India bedsores and gangrene of the fingers, toes, nose and ears have been reported especially in cases of the typhoid form. Disturbances of the eyes have also been noted due to disappearance of the lacrimal secretions and the appearance of secondary conjunctivitis. Ulcerations of the cornea have also been observed particularly in cases after prolonged collapse in part due to the lack of ocular secretions and to the fact that the eyes are kept half open and secondary bacterial infection occurs. The danger of sudden cardiac failure must at times be kept in mind. Pregnant women generally miscarry and the foetus itself may show evidences of cholera infection.

### DIAGNOSIS

**Clinical Diagnosis.**—The diagnosis of Asiatic cholera from the clinical picture in severe cases is usually easy during an epidemic. The sudden onset, the purging and vomiting of rice water like material, the extreme prostration, collapse of the circulation, the cholera facies, muscular cramps and complete suppression of urine constitute a very typical and striking clinical picture. However the diagnosis may be very difficult in mild cases or in sporadic cases which may precede an outbreak. Also the diagnosis may be confused with certain other intestinal disturbances. In such cases an accurate diagnosis can only be made by a bacteriological examination.



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#### COMPLICATIONS AND SEQUELAE

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will enable a correct diagnosis to be made. *Internal haemorrhage* may sometimes simulate the collapse stage of Asiatic cholera.

Manson Bahr also mentions the possibility of the early stage of *trichinosis* being sometimes confused with Asiatic cholera. When the adult worms in the small intestine reach sexual activity gastro intestinal irritation is produced. Abdominal pain, vomiting, severe diarrhoea of the choleraic type may ensue if the infections are severe with muscular cramps and pains. The presence of eosinophilia and the finding of trichinae would however establish the diagnosis.

Stitt (1909) points out that *bacillary dysentery* is sometimes difficult to differentiate clinically from cholera, and that many cases of cholera occurring in the Balkan War were diagnosed as bacillary dysentery. Manson Bahr (1939) refers to epidemics of the choleraic form of dysentery which were mistaken for cholera during the world war. The examination of the stool however will usually give definite information. In all severe cases of bacillary dysentery the amount of blood or mucus present is much larger than is seen in Asiatic cholera.

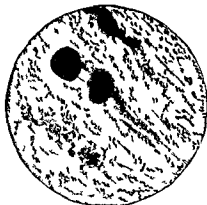


FIG. 53—Ch 1 a b — fish net trap (Aft. Jochmann f. m. May 1)

Acute *ar enical* and *m c ry poisoning* may also resemble cholera, but with these vomiting is usually the most striking symptom, though purging may also occur. Severe abdominal pain of a colicky character may occur and the stools may contain bile and blood. Also there may be a metallic taste in the mouth. As in cholera there may be a leucocytosis.

Manson Bahr points out that fireman's or stoker's cramp may perhaps cause confusion. It has been observed among those who work under conditions of excessive heat and moisture, such as are found in the engine rooms and stoke holes of ships in the tropics, especially in the Red Sea. The sufferers sometimes excrete frequent watery stools and suffer from marked collapse and severe muscular cramps. It is said that they may bear a considerable resemblance to cholera. Sellards (1936) has called attention to a somewhat similar condition among travelers in hot dry desert areas. The symptoms are brought about by excessive sweating and loss of chlorides, and treatment consists of giving large amounts of fluid at first containing 10 grms of sodium chloride to the gallon.

Finally Watson has reported several cases of infection with the trematode *Gastrophilus* which is met with in America principally in which there were acute intestinal symptoms ending in death. Two of the cases were diagnosed as Asiatic cholera. The correct diagnosis may be made by finding the ova of the parasite in the stools or the reddish translucent flukes themselves.

In all these instances obviously the bacteriological diagnosis should also differentiate Asiatic cholera.

Cases of Asiatic cholera may sometimes be confused with outbreaks of cholera nostras or different forms of food poisoning in which symptoms of acute gastro enteritis, diarrhoea abdominal pain, cramps, and vomiting may occur. The symptoms may occur almost simultaneously among a number of a group who have partaken of the same food as meat, milk cheese etc. The incubation period is usually short 6-30 hours, and its onset sudden.

Cholera nostras or outbreaks of food poisoning are usually due to infection with one of the organisms of the Salmonella group especially *S. enteritidis* or *S. aertryke*. The infecting organism can be isolated from the blood urine or faeces or in case of death from the viscera. However diarrhoea rarely lasts more than 5 days. The organism disappears from the faeces usually in from 7-10 days from the onset of symptoms. Specific identification of it can be made by the agglutination test as described in Chapter XVI where further details of the condition are described. However in some outbreaks of cholera nostras *Vibrio proteus* has been isolated. Groups of individuals have also been attacked. On a few occasions in the tropics the writer had opportunity to observe outbreaks of cholera nostras among garrisons of soldiers. The infection having occurred from eating partially decomposed meat. In the first of these outbreaks 73 individuals were attacked many with very severe gastro intestinal symptoms.

In food poisoning the blood serum of the patient usually also shows an agglutination reaction which appears in from 6-8 days after the onset of symptoms. This reaction might be of value in diagnosing cholera nostras and excluding Asiatic cholera in an individual who had been vaccinated recently against the latter disease and whose serum therefore would also show an agglutinating reaction against the cholera spirillum. A leucocytosis is present in the early stages of cholera but absent in food poisoning.

In cholera nostras and other forms of food poisoning faintness muscular weakness and prostration may be quite marked. Thirst is usually present and there is often a rise of temperature although many cases are apyrexial. The stools in outbreaks of food poisoning are watery but they are usually not so devoid of biliary coloring matter as they are in cases of severe cholera. However the milder cases of Asiatic cholera do not show rice water like evacuations and these may contain biliary coloring matter. Stitt has pointed out that these affections can at times show as marked muscular cramps emaciation cyanosis and a weak voice as in cholera and hence only the bacteriological examination can differentiate them.

In *botulism* the first symptoms may be gastric disturbances nausea and vomiting and occasionally diarrhoea. However there is no fever during the attack and while prostration is conspicuous and the onset is sometimes with vomiting obstinate constipation is the rule. Paralysis is also often the outstanding symptom. Difficult articulation and perhaps complete aphonia may be present with inability to swallow due to paralysis of the laryngeal and pharyngeal muscles. Rosenau points out that the most characteristic symptoms are dimness of vision diplopia palpebral ptosis fatigue progressive muscular weakness difficult articulation and swallowing and respiratory paralysis the clinical picture being essentially that of bulbar paralysis. Bearing these features in mind there should usually be no confusion with Asiatic cholera. However in doubtful cases the bacteriological examination will obviously clarify the diagnosis.

*Mushroom poisoning* is due to the ingestion of poisonous fungi of the genus *Amanita*. In the clinical variety which has been termed *Mycetismus choleraiformis* the symptoms consist of violent abdominal pains followed by nausea and vomiting and usually profuse diarrhoea. Severe hepatitis and jaundice may be present and toxic nephritis and anuria also result. However in mushroom poisoning the vomiting usually precedes the diarrhoea and the particles of the mushrooms may often be seen in the evacuations.

Rogers and Manson Bahr (1939) refer to certain forms of *algid* or *choleraic malaria* as closely simulating cholera. However the stools are never rice water like in character and high fever early in the disease and the presence of malarial parasites in the blood

ing of this approaching change. However observations on the reaction of the blood serum to phenolphthalein or determinations of the carbon dioxide of the blood or alveolar air will give suitable evidence. As these factors become nearly normal the dosage of bicarbonate should be reduced at least to about 10 grams. These figures apply only to the bicarbonate and not to the normal carbonate. Moreover precautions must be used in intravenous injection in the sterilization to prevent excessive formation of the carbonate.

### LABORATORY DIAGNOSIS

KEY to recognition of gelatin liquefying motile and Gram negative spiral or comma shaped organisms

I Give the nitroso indol reaction with sulphuric acid within 24 hours

(a) Very pathogenic for pigeons

(1) *Vibrio metchnikovi* (*Spirillum metchnikovi*) Liquefies gelatin about twice as rapidly as the cholera. Gives bubble appearance at top of stab. Produces an acute enteritis in fowls. Injection of culture into pectoral muscles of pigeons produces a fatal septicaemia. Not pathogenic for man.

(b) Scarcely pathogenic for pigeons

(2) *Vibrio cholerae* (*Spirillum cholerae*)

II Do not give the nitroso indol reaction (cholera red) with sulphuric acid alone in twenty four hours and furthermore especially in the case of Denecke's spirillum the cholera red reaction may be negative after prolonged cultivation

(a) Produce an abundant moist cream colored growth on potato at room temperature

(1) *Vibrio parvulus* (Finkler and Prior's spirillum) Liquefaction of gelatin very rapid. No air bubble appearance at top of liquefied area. Cultures have foul odor. Milk coagulated. Thicker and somewhat larger spirillum than that of cholera. Isolated from cholera nostras.

(b) Scanty growth or none at all on potato at room temperature. Only a moderate yellowish growth when incubated at about body temperature.

(2) *Vibrio tyroginus* (*Spirillum tyrogenum* Denecke's spirillum) Does not liquefy gelatin so rapidly as that of Finkler Prior. Milk not coagulated. Thinner and smaller spirillum than that of cholera.

NOTE.—Non motile non liquefying and Gram positive spirilla have also been described. There is also a large group of phosphorescent spirilla.

During the acute stage of the disease the vibrios can be demonstrated in large numbers in the rice water stools in films and by cultures. They do not penetrate into the submucosa and are not found in the blood.

The bacteriological diagnosis should be first undertaken in connection with epithelial flakes in the evacuations. If the vibrios are numerous they may be detected by their scintillating movement in a hanging drop preparation. In films from a fleck of mucus hardened and stained with carbol fuchsin 1 to 10 dilution their morphology as comma forms and appearance as fish in a stream is very characteristic.

Koch, in early years stated that during an epidemic a diagnosis could be made from the microscopical examination of the stool in half the cases.

However in regard to the morphology of the cholera spirillum it frequently shows a tendency to pleomorphism under different conditions and comma and spiral forms are not always present.

More rarely long straight rods or ovoid organisms of coccoid form occur and while motility is usually marked it may be almost absent.

In the diagnosis of *uraemia* in the later stages of the disease and in the typhoid like stage the subjective symptoms should be carefully considered

These may include headache dizziness disturbance of vision muscular twitching attacks of loss of consciousness nausea vomiting shortness of breath stinging of the skin fatigue lassitude and an erythematous rash Elevation of the blood pressure usually occurs In some cases there may be marked restlessness delirium twitching of muscles and generalized convulsions or the patient may pass into a comatose state

While the urine will contain albumin and casts this will often not aid particularly in the diagnosis because in all severe cases of cholera the urine contains both albumin and casts On account of the general condition of the patient in cholera functional tests of the condition of the kidneys which call for quantitative estimates of the diet are not practicable The phenolsulphonephthalein test is also usually unsatisfactory since it is frequently difficult to secure the necessary amounts of urine The blood urea nitrogen other nitrogenous bodies or the total non protein nitrogen in the blood may be determined These in uraemia are of course usually elevated and often markedly so However it is often impracticable to carry out these examinations in time to be of value except in regard to prognosis

The diagnosis of *acidosis* in Asiatic cholera is of particular importance with reference to the treatment In this condition it should be borne in mind that the origin of the acidosis is different from that which occurs in diabetes In diabetes the acids which accumulate in the tissue fluids are aceto acetic and beta-oxybutyric which are related to acetone and are derived from fatty acids and faulty metabolism In diabetes therefore foreign acids are added to the blood In acidosis in cholera and in the uraemia occurring in certain other acute nephritides from other infections the acids of normal metabolism accumulate particularly because of faulty excretion through the kidneys Possibly also other special and abnormal acids are developed in Asiatic cholera but this has not been demonstrated The usual signs of acidosis exist in both diabetes and Asiatic cholera because the surplus of acid depletes the stores of bicarbonate and causes changes in the alveolar  $\text{CO}_2$  in the  $\text{CO}_2$  absorbing power of the blood in the reserve alkalinity and in the acid excretion of the kidney As has already been pointed out acidosis in cholera often makes its appearance early in the stage of reaction and in cases showing the most profound evidence of uraemia There is then not infrequently a deficit in the body of fixed bases as is evidenced by the greatly increased tolerance to bicarbonate and the greatly decreased  $\text{CO}_2$  content of the blood In many cases an actual diminution of the body alkali occurs and the concentration of bicarbonate in the blood is reduced below the normal level The diagnosis of acidosis in cholera may be made from a determination of the  $\text{CO}_2$  tension of the venous blood or of the alveolar air but the reactions are often not practicable Selfards however has described a simple method of determining the tolerance of the patient to sodium bicarbonate The test may be carried out either by the ingestion or by the intravenous injection of bicarbonate For the detection of a slight increase in tolerance it is quite sufficient to give five grams of sodium bicarbonate by mouth every two or three hours until the urine becomes neutral or alkaline to litmus The bicarbonate should be given in a moderate amount of water and the patient should void before each administration Specimens of urine which are not distinctly acid should be boiled thoroughly to convert bicarbonate to carbonate so that it will react readily to litmus Intravenous injection may be required if abnormalities of the gastro intestinal tract exist and when large doses of bicarbonate become necessary Even in a normal person without any acidosis as much as 3 to 5 grams of bicarbonate can be given intravenously without discomfort and can be repeated at intervals of 10 or three hours until the reaction of the urine changes In marked cases of acidosis massive injections of bicarbonate are necessary to render the urine alkaline A very ordinary dose for intravenous injection would be one half liter of a 4 or 5 per cent solution repeating this dosage every 4 to 6 hours A liter of 5 or 6 per cent solution given slowly over a period of one half to one hour should be regarded as a maximal dose One must approach with some caution the point at which the urine is about to change over to an alkaline reaction Examination of the urine gives no warn

**Krumwiede's Medium.**—Krumwiede's formula is as follows. Take equal parts of whole egg and water and add to the mixture an equal volume of 12.5 per cent sodium carbonate (crystals) solution. Having steamed this alkaline egg mixture for twenty minutes add 30 parts to 70 parts of meat extract free 3 per cent agar. (No meat extract only peptone and salt.) The surface of the agar must be dry. The cholera colony has a hazy look like a little wad of absorbent cotton sticking to the surface with a metallic luster halo.

**Goldberger's Medium.**—First prepare a meat infusion by treating 500 grams of finely chopped lean beef with 500 cc water. After three hours strain the infusion adjust reaction to neutral with 5.3 per cent anhydrous sodium carbonate then add to each 100 cc 2.5 cc of 5.3 per cent anhydrous sodium carbonate solution sterilize in Arnold for one half hour and filter. Next prepare a 3 per cent meat extract agar and mix one volume of the alkaline meat infusion with 3 volumes of the hot melted 3 per cent meat extract agar. Pour plates and cover with a piece of filter paper and place in incubator for one half hour until they are quite dry. On this medium cholera grows well while faecal bacteria are restrained. The cholera colony is clear round and shows a brownish center but is without that striking bluish opalescence shown on ordinary agar plates.

**Esch Medium.**—This medium has been highly recommended. It is easy to make. Heat 500 grams chopped up beef with 250 cc normal NaOH solution in a pot and when disintegrated filter through cloth and sterilize. About 1 part of this alkaline extract is added to 2½ to 2 parts of nutrient agar. The plates must be dry. The transparency of this medium is an advantage.

**Aronson's Medium.**—This is an excellent medium for the examination of stools of cholera carriers. The organisms taken from such plates emulsify easily and there is no interference with their agglutinability. To prepare it add to 100 cc of 3 per cent nutrient agar 6 cc of 10 per cent solution of extracted sodium carbonate and steam in Arnold sterilizer for fifteen minutes. Then add 5 cc of 20 per cent saccharose solution, 5 cc of 20 per cent dext in solution, 0.4 cc saturated alcoholic basic fuchsin and 2 cc of 10 per cent sodium sulphite. A precipitate forms which quickly settles and plates can be poured from the supernatant fluid. Cholera colonies develop in 12 hours and show as red colonies in 15 to 20 hours. Colon colonies are much larger than these and are colorless. In stock cultures of the cholera vibrio the colonies are much slower in development.

Fead (1939) believes that a modification of the bismuth sulphite enrichment medium of Wilson and Blair is most satisfactory. The one is late the vibrios from an inoculum that would only just grow in ordinary broth. The bismuth sodium sulphite medium was modified by the omission of brilliant green, an increase of the pH to 9.2, the substitution of mannose for mannite in 1 per cent concentration and replacement of the broth by peptone water. By the use of this medium he states mannose fermenting vibrios can be successfully differentiated from non mannose fermenting vibrios and from polyform types. Other common water and stool organisms except streptococci are suppressed. However this method does not distinguish *V. cholerae* from other mannose fermenting nonagglutinable vibrios and hence the value of the method depends on whether these non pathogenic mannose fermenting vibrios can outgrow *V. cholerae* or not. It will be necessary for further use to determine whether this media is more reliable than that of Aronson for such differentiation.

Most cholera organisms when freshly isolated give the cholera red reaction. The test is performed by adding concentrated sulphuric acid free of nitrite to the peptone. The reaction is due to the fact that the cholera spirillum usually produces both indol and nitrites in the media. However during some epidemics from 5 to 10 per cent of the vibrios isolated may fail to give this so-called cholera red reaction. Some of the cholera vibrios produce a mild haemolysis on blood agar and others do not.

With reference to pathogenesis freshly isolated organisms usually show a high pathogenicity for guinea pigs upon intraperitoneal inoculation killing the animal in doses of one tenth to one half loop but occasionally the pathogenicity of the cholera organism may be very slight for guinea pigs. On the other hand certain vibrios from water may also show pathogenesis for guinea pigs.

During one of the recent epidemics of cholera in Japan, two strains of the cholera vibrio were encountered, one of which was reported as showing no motility whatever

If numerous motile spirilla and stained comma forms are found respectively in the fresh and hardened preparations while the diagnosis of an isolated case of cholera may be suggested, a definite diagnosis should never be made on the simple microscopic examination alone because we now know that spirilla are sometimes found in many cases of non-choleraic diarrhoea and sometimes even in the stools of normal individuals

For the isolation of the cholera organism a number of selective media have been prepared and recommended. These selective media while they exert a restraining influence over the growth of many of the intestinal bacteria permit the growth of the cholera spirillum

The media recommended by Dieudonné and its various modifications by Goldberger, Krumwiede, Aronson, Esch and others are of considerable value in this connection.

Dieudonné's medium which has been given a thorough trial with good results consists of a mixture of equal parts of defibrinated blood obtained at the slaughter house and normal NaOH solution. Mix 30 parts of this alkaline blood mixture with 70 parts of hot 3 per cent nutrient agar. The poured plates must be left half open over night in the incubator to dry otherwise even cholera will not grow on them.

Upon the media of Dieudonné drops of the intestinal evacuations are inoculated with the bacteriological needle by streaking upon the surface. The cholera vibrio which shows a special toleration for alkali grows abundantly on this media. On the other hand organisms as *Bacillus coli*, the bacillus of typhoid fever, paratyphoid bacilli or very importantly the bacillus of dysentery grow either very slowly or not at all. On the contrary the noncholeraic vibrios of water and faeces and *Bacillus proteus* and *pyocyaneus* and several other organisms behave very nearly like the cholera vibrio. Both *Bacillus proteus* and *pyocyaneus* are encountered in diarrhoeal disturbances very frequently and hence these organisms complicate the search for the cholera spirillum. The cholera colonies usually appear on the media after from 6 to 8 hours. Plate cultures should also be made from the stool upon slightly alkaline agar.

With the isolated colonies which appear on the plates within 24 hours the agglutination test may be performed with a stock cholera immune serum. In favorable cases the cholera organism can be isolated and identified by the serum reactions in less than 24 hours. In instances where few cholera organisms are present as is frequent in the late stages of the disease in convalescence and in cholera carriers it is better to make a preliminary enrichment of the culture by inoculating with a loop of the faeces into a tube of alkaline peptone (pH 8-9). In this medium the cholera vibrio multiplies rapidly at the surface so that a pure culture may often be obtained by removing a loopful from the top after 3-8 hours. Stained films may be made of such a loopful of the preparation examined in which characteristic motile vibrios may be present. Another loopful is then spread on a Dieudonné plate or on a plate of alkaline agar. After isolation the organism is identified further culturally.

On gelatin plates the cholera organism produces a rapid liquefaction of this media. Gelatin however is rarely used in modern bacteriological laboratories. On agar plates the cholera colonies appear flatter of greater delicacy more transparent and grayish blue in color while the colonies of the other intestinal organisms, as *Bacillus coli* are more globular and opaque. The following other selective media for cholera have been recommended.

experiment is made upon material where vibrios are found mixed with an abundant and varied microbial flora it becomes difficult if not impossible to count upon results of any value

However when colonies are present on solid media it is possible to carry out microscopical agglutination tests by suspending a portion of the colony directly in a loopful of suitably diluted immune serum (1-500 or 1-1000 of a high titer serum). A hanging drop preparation is examined under the high power for agglutination and loss of motility. A suspension in salt solution is made as a control. Subcultures should be made from the same colonies for further studies.

However the most satisfactory method for a bacteriological diagnosis of the cholera vibrio is by the macroscopic method in the test tube.

In this method one bacteriological loop of the living organism from a pure 24 hour slant agar culture is thoroughly suspended in one cubic centimeter of an 0.85 per cent solution of sodium chloride. The amount of serum to be tested suspended in one cubic centimeter of a similar saline solution is then added, the tube thoroughly shaken and the mixture placed for 1 hour at 37°C. In a complete agglutination the organisms are deposited at the bottom of the test tube and it is understood that the liquid overlying the precipitated bacteria appears entirely clear. By a weak reaction we understand one in which there is a distinct agglutination with precipitation visible to the naked eye of numbers of the organisms but in which the supernatant fluid remains more or less cloudy. In Asiatic cholera the reaction should occur in dilutions of 1:1000 to 1:10000 with a satisfactory agglutinating serum. These sera for diagnostic purposes are prepared commercially in sealed tubes and it is only necessary to perform the test to dilute the dried serum in sterile distilled water and note the necessary dilutions. A satisfactory serum can also be obtained by the intravenous injection of a rabbit with a suspension of  $\frac{1}{2}$  a loop (of a 2 mm needle) of a culture of the cholera vibrio and the bleeding of the animal after 8 days.

Some strains may be inagglutinable when first isolated as is sometimes the case with the typhoid bacilli.

For the bacteriological diagnosis of the cholera vibrio in the laboratory Pfeiffer's phenomenon which depends upon the bacteriolysis of the cholera vibrio in a cholera immune serum in the abdominal cavity of the guinea pig is the most satisfactory and accurate reaction. The method of performing this reaction is given on p. 601. This bacteriolytic reaction of the cholera vibrio may also be performed by the method of Bordet in the test tube but is less satisfactory.

From time to time vibrios have been isolated from the stools or from the intestines of individuals who have suffered with diarrhoeal attacks or cholera like attacks. These vibrios have resembled the true cholera vibrio in many respects but have not given the agglutination reactions for the cholera vibrio. They have differed in minor characteristics and a number of them were not agglutinated by an anti cholera O serum. Sometimes they have been designated as para cholera vibrios. The question has arisen are some of these vibrios cholera vibrios and are they capable of giving rise to epidemics of Asiatic cholera? There is not yet unanimity of opinion on the question.

Linton (1940) believes the cholera organism should possess the following combination of characteristics: fermentation of mannose and sucrose but not of arabinose; failure to haemolyze goat's blood; and agglutination with O group I serum according to Gardner and Venkatraman (see Table p. 598).

Pasticha (1939) and his associates have studied the development of the H and O agglutinins in the serum of cholera patients. The blood from 175 cholera patients



While the cholera spirillum is usually found in abundance in the stools during the acute stages of the disease and sometimes almost in pure culture in the later stages it becomes greatly decreased. The other bacteria in the intestine then predominate and it is very difficult or sometimes impossible to detect its presence. In the dejecta of individuals who have had an attack of cholera the vibrio may persist after complete recovery in about one third of the cases for as long as 10-14 days. In very rare instances it has persisted longer or from 50 to 100 days.

*For isolation of the vibrios from water* 900 cc. is added to 100 cc. of 10 per cent peptone solution containing 5 per cent NaCl. This mixture is distributed into a number of sterile flasks and after 24 hours the surface growth is examined and subcultured.

**Diagnosis at Necropsy**—At the necropsy of cholera cases the vibrio is met with in abundance in the small intestine in the layers of desquamated epithelial cells and mucus which cover the surface and frequently in the large intestine. It sometimes is found even in the superficial layers of the lymph follicles of the small intestine. It is usually not found in other organs and not in the blood. In a few instances its isolation has been reported from the gall bladder. Greig of India has also reported its occasional presence in the urine of fatal cases and even very rarely in the lungs. In the latter place the infection is very rare and was perhaps secondary from inspiration of the infected vomitus. Isolation of the cholera vibrio from either the urine or lungs has not been reported in recent years.

**Immunity Reactions**—It should be emphasized that morphological characteristics, motility, cultural characteristics, and animal experiments alone do not enable us to arrive at a certain bacteriological diagnosis of the cholera vibrio since there have been isolated from various sources (notably different water supplies and the normal intestine) a number of vibrios which cannot be distinguished from the cholera vibrio by these reactions and yet which are not capable of producing cholera.

For this differentiation it is necessary to employ the immunity reactions (1) the agglutination reaction and (2) the bacteriolytic reaction in vivo or the Pfeiffer phenomenon.

The *agglutination test* as a means of diagnosis in Asiatic cholera is most valuable in connection with the identification of the cholera vibrio isolated from the evacuations. It however may be employed with the blood serum of the suspected case and a known culture of the cholera vibrio. However the agglutination test performed with the patient's serum is not satisfactory for the diagnosis of Asiatic cholera in the early stage of the disease and even in fatal cholera cases the serum rarely gives a positive agglutination in a dilution higher than 1:40. In cases which are recovering after the sixth day the agglutination test made with the serum of the patient may be positive in dilutions as high as 1:500 or 1:1000. This agglutinating power of the serum falls rapidly after the third week but may be still present after 6 to 8 months. The serum of individuals who have been vaccinated against cholera also gives the agglutination test sometimes in dilutions as high as 1:2000.

Dunbar has recommended a quick diagnostic agglutination method for the identification of the cholera spirillum in the evacuations. A flake of mucus or a drop of the dejecta is suspended in several drops of peptone solution. On one cover glass is deposited a drop of 1:50 normal serum and on another cover glass a drop of 1:500 dilution of cholera immune serum. A loopful of the suspected stool suspension in the peptone solution is then rubbed up in each of these drops of serum the two coverslips inverted over hanging drop slides and examined under the microscope. When the material examined contains as often happens in cholera a pure or almost pure culture of cholera vibrios on examining microscopically the two drops comparatively it is seen that in the preparation which contains the drop of cholera immune serum there is often a prompt clumping and cessation of motility of the cholera vibrio while in the preparation containing normal serum the vibrios remain actively motile and isolated. When the

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was examined the agglutination test was made in Dreyer's tubes using a formalized 0.2 per cent suspension of a young agar culture as the H antigen and a boiled suspension in saline as the O antigen. Alcohol treated vibrios or vibrios suspended in 0.5 per cent lithium chloride were found to be not as satisfactory as the boiled suspension. Final readings were taken after 18 hours in a water bath at 55 C.

They conclude that H agglutinins appear early and in a larger percentage of cases than O agglutinins. Agglutinins are better developed for the homologous strain than for the standard *V. cholerae* (Inaba). In a few patients they found H agglutinins develop for the standard strain of *V. cholerae* and not for the homologous agglutinable strain. In the serum of mixed cholera cases i.e. passing *V. cholerae* and inagglutinable vibrios agglutinins were demonstrable only for *V. cholerae* and not for the inagglutinable strains. In the serum of cholera patients from whom vibrios were not isolated, from the stools agglutinins for *V. cholerae* were nevertheless found. They stress the importance of serological tests in the retrospective diagnosis of cholera in those patients in whom *V. cholerae* has not been isolated from the stools during the acute stage of the disease. Pasricha (1939) states that in 15 patients in whom repeated stool examinations had yielded negative results in 5 agglutinins for *V. cholerae* were present in the blood with both Inaba H and O antigens.

It has recently been suggested (1939) that the so-called Ogawa strain of the cholera vibrio might only be a strain developed in the laboratory by artificial culture and not a vibrio occurring in nature. However Shortt and Pandit at the King Institute of Preventive Medicine at Guindy reported in 1937 that several hundred Ogawa strains had been encountered and it has been found that the 2 main cholera types termed Inaba and Ogawa have been met with in special areas or zones in which one or the other predominate. There was however in 1938 no difference in the severity of the epidemics caused by either the Inaba or the Ogawa type. In Japan the Inaba type as intimated has been regarded as the original type of cholera vibrio the Hikojima as the middle type and the Ogawa as the variant type. These types were isolated 15 years ago and it is said by Nishimura (1939) that they still retain their specific character. Sixteen strains he tested recently proved to be of the original Inaba type.

Pasricha reports that at the School of Tropical Medicine Calcutta during the first half of the year 1938 agglutinable vibrios were isolated from 58 per cent of the samples of faeces from clinical cases of cholera and non agglutinable vibrios were isolated from 7 per cent. Vibrios were not isolated from 35 per cent of the cases. During July, August and September vibrios were isolated from 16 per cent of the cases. Vibrios of the Ogawa type were found in 34 or 13 per cent of 250 patients studied. From 15 of these patients both the Ogawa and the Inaba types of vibrios were isolated. The results of such studies are of greater value with reference to bacteriological diagnosis if one knows definitely the day of the disease on which the examination was made also the reaction of the cholera stools is important.

**The Reaction of Cholera Stools**—In a study of the hydrogen ion concentration of stools of 150 cholera patients collected during the first 2 days after the onset of symptoms by the calorimetric method approximately 65 per cent of typical rice water stools without visible faecal matter were alkaline. The majority of these samples had a pH value between 7.5 and 8.5. About 35 per cent of the stools were acid (pH below 7.0 but not below 6.0). Stools containing faecal matter were distinctly acid in reaction. In 30 cholera patients whose stools were examined daily vibrios were found in nearly all the alkaline samples and in fewer samples when the reaction of the stool was on the acid side.

## PROGNOSIS

There is the greatest variation in the mortality in different epidemics as is true of most other epidemic diseases. In the early stages or at the peak of the epidemic the mortality usually is highest. In untreated cases it has reached 80 per cent. However 50 per cent may be considered an average mortality. During epidemics when many patients are given

treatment late in the stage of the disease Sellards (1936) estimates that about 30-35 per cent die in the stage of collapse and that it may be expected that 15 per cent will die of uraemia unless intensively treated with alkalis. Under more favorable circumstances when patients are treated early the mortality may be as low as 25-30 per cent.

In young children and old people there is usually a very high mortality rate as is also true of alcoholics and those with kidney disease. The mortality is also high with pregnant women.

### PROPHYLAXIS AND TREATMENT

**Prophylaxis**—Personal prophylaxis is apparently of greatest influence in protection from infection. In this connection it is important to consider the way in which cholera is contracted and spread. (See pages 604-613.)

For the prevention of cholera during an epidemic two factors are of paramount importance: first the protection of water and food supplies and second the destruction of excreta of patients.

In the presence of cholera one should only drink recently boiled water which has been protected from the contaminating influence of flies and all forms of uncooked food should be avoided. Important among prohibited foods should be raw shell fish and uncooked salads. Such articles as lettuce and celery are particularly dangerous on account of the moisture retained. Fruits such as bananas and oranges can be made safe by covering them with boiling water for two or three minutes and subsequently peeling. Care must be taken that native servants do not put fish which may have been contaminated with cholera infected water on the ice in an icebox and through such a source to have the butter etc. infected. The most scrupulous attention should be given the matter of the care of the ice box in the tropics.

All drinking water and all water used in washing of dishes should be boiled. In emergencies where it is impossible to boil the drinking water it should be carefully chlorinated and the chlorination controlled to show if sufficient free chlorine is present to destroy the bacteria. The routine procedure with bleaching powder 1.3 per cent of chlorine per million or 2 grammes to 110 gallons of water cannot always be relied upon as safe. Sodium bisulphate tablets 2 grams to  $1\frac{3}{4}$  pints of water by liberating sulphuric acid are useful for sterilizing water in canteens on the march. Disinfection of the drinking water in the wells in rural communities should receive special attention and sterilization of wells by chlorination is usually considered more satisfactory than is the addition of potassium permanganate (60 gr. to the gal.) which has been especially recommended in India. The permanganate appears to be of value in precipitating the organic matter in suspension rather than by actually killing the bacteria. The value of the use of bacteriophage in destroying the cholera spiroillum in wells is still speculative and should not be relied upon.

Besides care of the food and water ingested particular attention should be paid to the washing of the hands before eating and if one has been in

contact with cholera cases there should be careful disinfection of the hands

Tea has been recommended as a prophylactic and its use is advisable as it implies boiling of the water. Eucalyptus oil has also been advised as a prophylactic, 10 minims twice daily. As acids have an inimical effect on the cholera spirilla some have recommended the use of acid drinks but as a matter of fact the best prophylactic is the normal gastric juice, and there is a possibility that the use of such acid drinks might upset the digestion and defeat the object desired.

Experience in cholera epidemics has shown the importance of avoiding anything which might lower the resistance. Fatigue, excess in alcohol or the taking of any kind of indigestible foods are to be avoided. It must be remembered that the use of purgatives may set up cholera in a cholera carrier so that this possibility should be thought of.

An important municipal measure for the control of a cholera outbreak is the diagnosing of cholera carriers, such cases often occurring in those associated with a cholera case. Such carriers should be isolated and their stools disinfected until at least 2 negative examinations show them to have ceased being cholera carriers. Of course a cholera case should be isolated and kept in a fly screened room. All autopsies should be performed in a fly screened morgue.

Other municipal measures should include

1. Improvement of the water supply
2. Provision for treatment and isolation of patients
3. Discovery and notification of cholera cases
4. An educational campaign
5. General sanitary improvement of the district
6. Protective inoculation

In China recently (1939) it was found necessary to pay special attention to sterilization with chlorine of the water buckets used to transport drinking water.

For disinfection of stools one may employ an equal amount of a 5 per cent compound cresol solution which when mixed with the same amount of stool becomes a 2½ per cent solution. This should be in contact with the stool at least one hour before emptying the container. Chlorinated lime 1 pound to 4 gallons makes an excellent disinfectant for stools—equal parts of this 1 to 16 chlorinated lime solution and stool.

Bed clothing or other material contaminated by vomitus or faeces should be immersed in a 2½ per cent compound cresol solution. All food utensils should be disinfected by boiling. Persons attending cholera cases should wear gowns and remove the same upon leaving the room. Particular care should be exercised in hand disinfection after attending a cholera case.

There is no danger from aërial conveyance of infectious material other than the possibility of one's coming within the danger zone of a vomiting patient. Therefore for disinfection of a room occupied by a cholera patient we need not use formaldehyde gas but washing of floors and lower part of walls with 2½ per cent compound cresol solution is sufficient. The stock solution of chlorinated lime 1 pound to 4 gallons is suitable for mopping floors and walls.

**Quarantine**—When cholera was epidemic in parts of Europe in 1911 at the New York Quarantine Station the cholera vibrio was isolated from 28 immigrants arriving on steamers sick with the disease and 27 healthy persons were found to be infected with the vibrios in their faeces. Seven cases of cholera were detected at other ports by bacteriological examinations. The authorities believe that there was no doubt that the adoption

of these measures kept cholera out of the country. Undoubtedly also the introduction of cholera into the Philippine Islands from China has repeatedly been prevented through quarantine. Obviously in many instances it may be difficult to detect cholera carriers. However apparently no epidemic in a country has been started by any individual who has been a cholera carrier of more than two months standing.

During 1937 cholera was wide spread in China and in most of the important ports. From July 26 to August 24 802 cases and 427 deaths were reported in Hongkong and over 500 cases (in that city) from the latter date to September 11.

To prevent the introduction of the disease into the Philippine Islands the United States Public Health Service directed all quarantine officers to carry out very careful inspection of ships passengers and crew from infected or suspected ports including bacteriological examination to detect carriers. There was close cooperation with the public health service officers stationed at Hong Kong the Chinese Quarantine Service and the Eastern Bureau of the League of Nations with headquarters at Singapore. These cooperative efforts have been particularly directed towards the prevention of embarkation of infected persons.

It has been thought probable that the west coast seaports of the United States are not likely to become infected for the reason that since the incubation period of cholera is usually not beyond 5 days outbreaks on shipboard will occur and the disease will become evident some time before the ship from an infected port could reach a United States port. However the possibility of introduction of the disease by carriers is not being overlooked and a bacteriological survey is being conducted for carriers whenever indicated. Ships from cholera infected areas are not granted *radio pratique*. Passengers from infected areas travelling by Pan American Clipper air ships have not been inconvenienced since they have completed the incubation period by the time they reach San Francisco but if they stop over en route they are held at stop-over points to complete the incubation period. Since protected water supplies and protected milk supplies prevail in American cities today cholera is no longer the menace to the country that it was in earlier years.

### PROPHYLACTIC INOCULATION

With reference to protective inoculation against cholera it is well to recall that there is no general invasion of the body or of the blood by the cholera vibrio. The organism remains localized in the intestine and the symptoms are presumably due to the action of the vibrio in the intestine and the absorption of the toxin. Subcutaneous inoculation of killed cholera organisms into man gives rise to agglutinins and bacteriolysins in the blood but there is no evidence of antitoxic substances being produced by such inoculation. In fact with the cholera vaccines in common use today it is said the inoculation gives rise usually to no systemic reaction whatever in the individual. This in itself implies that little toxin has been introduced. Therefore from a theoretical standpoint it is questionable whether by inoculation the production of a small amount of bacteriolysins and agglutinins in the blood of the individual can either prevent an infection with the cholera spirillum or the progress of the disease for the cholera spirillum is not brought into direct contact with the blood serum as the typhoid bacillus is when it invades the blood and tissues in a case of typhoid fever.

While prophylactic inoculation in cholera has been very widely employed for many years, no definite unanimity of opinion exists in regard to its value during an epidemic although in general the reports have been favorable regarding its use. There is very great difficulty in assessing the value of cholera inoculation from the reports that have been published. Certainly in the great majority of outbreaks there has been no unvaccinated group living under exactly the same circumstances as the vaccinated group with which to make comparison of the results. Anyone who has had wide experience with cholera and cholera epidemics can hardly give credence to the mere statement that an epidemic entirely subsided as the result of inoculation.

**Methods of Inoculation**—Ferran (1885) first introduced bacterial vaccination of man in connection with a cholera epidemic in Spain. He demonstrated that guinea pigs could be protected against lethal doses of the cholera spirillum if they had been previously inoculated subcutaneously with small doses of the organism. Some 50,000 people were inoculated subcutaneously with living cultures of the cholera organism during two years but there were numerous accidents apparently some at least due to an impurity of the cultures and the vaccinations were stopped by the Government and their value was not demonstrated.

Haffkine in 1893 employed vaccination in India using a preliminary subcutaneous injection of an attenuated cholera organism and later a second injection of a more virulent culture obtained by repeated passage through animals following the principle of Pasteur's method of vaccination against anthrax. Later only the virulent culture was employed.

Kolle recommended as a prophylactic the cholera spirillum grown on nutrient agar suspended in sodium chloride solution 0.85 per cent and killed by heat for one hour by exposure to heat at 53–58 C. It was recommended that at least two inoculations of this prophylactic were advisable to produce a satisfactory immunity the first dose being 0.5 and the second 1 cc. after a week's interval.

Besredka has recommended a sensitized vaccine obtained by shaking the cholera spirilla with cholera immune serum. Later in 1922 he introduced his bili vaccine for oral administration. This vaccine is now made from thick suspensions of the cholera organism killed by heat, carbolic acid or alcohol and given in from 3–5 doses ranging up to 100 cc. every other day. Each dose is said to consist of 10–100 milliards of vibrios or 0.01–0.1 gm. of the dried organisms.

The writer (1902) working in Koch's laboratory with Wassermann prepared a cholera prophylactic consisting of filtered suspension of the immunizing substances of the cholera spirillum in normal saline solution which had been extracted and digested from the cholera spirillum. The extract was then sterilized by heat at 58 C. and preserved in 0.5 per cent carbolic acid. This form of prophylactic is obviously much more difficult to prepare but had the advantage that a larger amount of the immunizing substances may be given at a single time than it is possible to inoculate into man when the killed organisms are employed. A single inoculation is sufficient to produce a high immunity in animals and there is practically no local reaction in man. This prophylactic was used in different outbreaks in the Philippine Islands in 1903–7 the number of cholera cases among the inoculated being about  $\frac{1}{8}$ th of those that occurred among the uninoculated.

Pasricha and Chatterjee (1939) recently examined 6 cholera vaccines used in India from recognized laboratories. It was stated that all gave satisfactory antigenic response and protected guinea pigs against 2 MLD of *Vibrio cholerae*. (This represents a very low immunizing power since a potent vaccine should immunize guinea pigs against at least 10 times the MLD.) Four of 8 commercial preparations of cholera vaccines examined produced cholera agglutinins and gave rise to protective properties and 4 vaccines gave uniformly negative results. Kingsbury (1939) director of the Institute

for Medical Research of the Federated Malay States states that the cholera vaccine prepared at that institute contains Inaba and Ogawa subtypes in approximately equal proportions with the idea of providing the various antigens required to produce a satisfactory immunization response.

Yu (1938) emphasizes a most essential point as was demonstrated by the writer in 1903 that in the preparation of cholera vaccine the strain selected should have the highest possible virulence in the animal test as it was conclusively shown that the immunizing power of cholera strains is in proportion to their virulence.

**Results**—In recent years only killed cultures of the organism have been employed for prophylaxis during epidemics. During the Great War many inoculations were made. The initial dose recommended was  $\frac{1}{2}$  cc. of an emulsion of four thousand millions followed 7–10 days later by a second inoculation of 1 cc. containing eight thousand millions. In some instances oedema and a painful infiltration at the site of the injection occurred but this was rarely followed by systemic disturbances. In general, the subcutaneous inoculations were easily tolerated.

It was emphasized that the vaccine is not effective if kept longer than 3 months and that the protection is of comparatively short duration as only about 6 months. Indeed Manson Bahr (1936) says the immunity is regarded as lasting at the maximum for 3–4 months.

The reports of the results obtained in India and during the Balkan and World War have been on the whole favorable but comparisons of the actual value of the inoculations have not been conclusively demonstrated. In general statistics have shown that the case rate among the inoculated and the uninoculated has varied in different localities from 1 to 4 to 1 to 8 or 1 to 10 and the mortality among the inoculated and the uninoculated as about 1 to 2.

During the past few years cholera vaccination has become very popular and has been used on a wide scale in India, China, Indo-China and the Malay States.

In Haipong 1938 the outbreak of cholera was a severe one and its extent increased by the influx of refugees as a result of the Japanese attacks during the war. After the epidemic had been in progress for some months vaccination was begun on Sept. 3, 1938 and in 10 days by the 3rd of Oct. 94,000 persons had been vaccinated. Later the number of vaccinated was increased to at least 58,000. By the 13th of October there was a steady decline in the cases observable. Of 564 patients admitted to the hospital 94 per cent had not been vaccinated. Too much credence should not be given to the use of the vaccine in this connection for it is well known that in many parts of China it is customary for the outbreaks to reach their peaks in September, die down in October and disappear at the end of November.

It has been pointed out however that vaccination was not used in the Tonking Delta and the disease continued to prevail there after it had been extinguished in Haipong. However Quenardel (1938) reports that in Tonking a very active campaign of vaccination against cholera was introduced and 6,500,000 inoculations were performed in the short time of 4 months. Many reports were also received of the efficiency of the vaccination there but these reports were by no means all favorable. It was noted that the epidemic ceased in some non-vaccinated villages and that there was a recrudescence in some vaccinated areas. In the Haipong outbreak while the vaccine was thought to reduce the prevalence of the disease no evidence was obtained of its mitigating the severity of the attack if infection did occur.

Vogel and Riou who have examined the results of vaccination in Tonking saw no advantage in it. They report that 64,300 vaccinations were performed but that the medical officer in charge also observed spontaneous arrest of cholera in non-vaccinated villages and its recrudescence in vaccinated villages. They consider that epidemic cholera came to an end in non-vaccinated China at the same time that it did in vaccinated Tonking.



In the Province of Annam little difference in mortality was manifest between vaccinated and non vaccinated villages while in the Province of Vinh where cholera ceased in the primary focus after a massive inoculation of the population it was found that on recurrence it ravaged indifferently vaccinated and non vaccinated villages. They emphasize that it is necessary to be very circumspect in interpreting the results of vaccination in a favorable as well as an unfavorable sense.

Genevray and his associates (1938) reported an epidemic in Indo China in a Tonking delta village. The epidemic had a sudden occurrence in the community of not more than 1200 persons. No cholera had been notified in the province for 4½ months. It was all over in 15 days. There were 60 cases with 52 deaths. The vibrios isolated from the stools were of the Inaba type. A vibrio of this type was isolated from 2 village compounds but not from the only well of the village. There were no carriers found. Almost immediately the epidemic broke out 980 vaccinations were performed and they think that the sudden end of the epidemic was due to vaccination. It is not stated whether any further cases occurred among the unvaccinated population. Later however they reported that 13 513 000 vaccinations were carried out in Indo China. Apparently the epidemic did not end so suddenly elsewhere though it was reported the vaccination with one dose seemed to be efficacious. Nevertheless the mortality of the epidemic was high 68 per cent which is above the average mortality during epidemics. It was said however that when full vaccination was carried out as in the case of administration and military groups complete protection was obtained. It is presumable that the individuals of such groups also took all possible other precautions to avoid infection. *Monson and Rice (1934)* report that of 69 persons who were vaccinated and contracted cholera the mortality was 57.9 per cent and of 14 vaccinated 7 days or more before they fell all 8 died.

Shortt believes that in the evacuation from Burma owing to vaccination of the refugees and members of the labour battalions a threatened epidemic of cholera never assumed serious proportion. Over 50 000 were inoculated. In 150 000 refugees of which 95% were inoculated there were only 2 cases of cholera. In the Madras Presidency (1914-1944) approximately 4.4 million persons were inoculated in 1942. In a group of 140 745 inoculated there were 149 cases of cholera. In the group of 94 808 uninoculated persons there were 3 393 cases thus the incidence in the uninoculated was 10.9 times the rate in the inoculated. In the epidemic of 30 000 cases the mortality was 16 400. The mortality in the inoculated was 46.7% and in the uninoculated 63.5%. 2 637 cases occurred among the inoculated emphasizing that the inoculation is not always protective.

**Immunization by the Mouth**—This method recommended by Besredka in 1922 has been employed in Indo China and in the Malay States as well as in Russia. A mixture of the dried vaccine is known as bili vaccine and is made up into pills. Generally they are harmless, but sometimes they give rise to diarrhoea. The vaccine is made from dense suspensions of the cholera organisms killed by heat carbolic acid or alcohol and is given in from 3 to 5 doses ranging up to 100 cc every other day. Each dose consists of 10 to 100 milliards of vibrio or 0.01 to 0.1 gm of the dried organisms.

Vickers in 1928 in the Malay States made a comparison between the use of the usual vaccine given subcutaneously and Besredka's bili vaccine administered orally.

1. **Ordinary Subcutaneous Vaccine**—One dose only (0.5 cc = 4000 million organisms) given to 17 160 persons. Of these the percentage attacked was 0.34 and the fatality rate among those attacked was 37.3 per cent.

Two doses (together 12 000 millions) 8483 persons. The percentage attacked was 0.37 fatality rate 6.5 per cent.

Not inoculated 29 254 percentage attacked 1.67 fatality rate 37.6 per cent. Thus the relative numbers of cases among the unvaccinated was 4.5 times as large as among the vaccinated and the percentage case mortality as compared with that among those receiving the 2 doses was as 5.8:1.

2. **Besredka's Bili vaccine Orally**—The full course of 3 doses (= 200 000 million organisms) was given to 4982 persons the percentage attacked the case

mortality was 22.2 per cent. Of 11,004 untreated 2.02 per cent were attacked and the fatality rate among these was 41.9 per cent. The number of cases among the unprotected controls was therefore 5.6 times that among the vaccinated and the fatality rate was nearly double.

It would appear from these figures that the full course of the bi vaccine confers practically the same degree of protection as the ordinary vaccine administered subcutaneously but the immunity conferred by a single inoculation is nearly as high as that from the full course of the bi vaccine. The fatality rate however was 1.7 times as great after a single inoculation but only one third when the full dose was injected as compared with the oral bi vaccine.

Scott points out that the difficulty of interpreting satisfactorily and fairly the result of vaccinations used prophylactically where 2 doses are given is made greater by the fact that an outbreak may die down spontaneously by the time the second dose of vaccine is given.

Russell (1927, 1934) has also made a comparative test of the results obtained by the use of anticholera vaccine given subcutaneously and of the oral use of bi vaccine. He considers that in the presence of a cholera epidemic there would be an objection to the administration of oral vaccine on the ground that it might increase susceptibility to the bi vaccine pills at times caused an acute diarrhoea.

In 1448 villages who were given 2 doses of anti cholera vaccine there occurred 6 cases and 1 death but in 3083 persons who received 3 doses of bi vaccine 15 were attacked and 4 died.

It is reported that in an epidemic in Tokyo where oral vaccine was carried out at the height of the epidemic there were only 3 cases among 300,000 people vaccinated while in the 3,000,000 unvaccinated more than 600 cases were noted. Such statistics however are of little value without many details. Nothing is stated as to whether the vaccinations were performed among the more intelligent and upper classes in the neighbourhoods or whether all classes of people were inoculated.

The value of the oral method must still be regarded as *sub judice*. The value of bacteriophage in prophylaxis is discussed below.

The United States Army vaccine now being distributed consists of a suspension of 8,000 million killed cholera vibrios (*V. comma*) per cubic centimeter. The initial vaccination consists of two subcutaneous injections of cholera vaccine with an interval of from seven to ten days between the injections. The first dose 1.05 c.c. and the second dose 0.5 c.c. of the vaccine. A stimulating dose of 0.5 c.c. of cholera vaccine should be administered every four to six months as long as serious danger of infection is present. Other 0.5 c.c. doses of cholera vaccine may be given whenever in the opinion of the surgeon this additional stimulation of immunity is indicated.

#### THE VALUE OF CHOLERA BACTERIOPHAGE IN PROPHYLAXIS AND TREATMENT

It has been pointed out by D. Herelle that bacteriophage is a therapeutic principle which develops in the infected individuals and lies at the basis of natural cure. Applying this belief to the course of an epidemic its rise is due to the importation into a community of the cholera spirochillum which is spread by various agencies such as water, food, flies, etc. Individuals are infected and convalescents develop and excrete bacteriophage which is spread in a similar way and as more and more patients recover so the more widely is the phage disseminated and the epidemic brought to an end.

D. Herelle studied in the Campbell Hospital, Calcutta, 73 cases, the patients being examined for bacteriophage on arrival 10 to 20 hours after the onset of the illness. It was reported that those having no bacteriophage died in a few hours. In 1921 he failed to isolate phage in 10 cases in Indo China all of which died. Two however who were passing very active phage although very ill both recovered. In 3rd phage was weak and became weaker until it disappeared and all 3 died. In 13 it was reported weak at first but rapidly became stronger attaining a maximum in 24 to 48 hours. All

these recovered. In 1930 he and his associates investigated the question on behalf of the Government of India. In a total of 198 cases of cholera 74 received bacteriophage treatment of whom only 6 died while in a series of 124 cases not receiving the phage treatment the mortality rate was 78 per cent.

Morison and Lardon (1939) used a combined dysentery cholera bacteriophage in two epidemics of cholera in Assam. The mortality in the cases receiving no bacteriophage was 75.8 per cent while in the cases receiving bacteriophage treatment it was 9 per cent. Usually they employed 2 cubic centimeters of phage given 4 times daily by the mouth while serious cases received cubic centimeters intravenously in hypertonic solution.

On the other hand Souchard (1930) failed to obtain any benefit from the use of bacteriophage and in his series of cholera cases treated with it there was a mortality of 24 out of 27.

Taylor (1938) reports that results obtained in Calcutta show no appreciable difference in the death rate of cases treated by bacteriophage and the control series but that a certain value attaches to bacteriophage treatment when it is added to ordinary methods of treatment when only the figures for cases in which agglutinable vibrios were isolated from the stools are taken into account.

Pasricha (1939) reports that at the Campbell Hospital at Calcutta altogether 272 cholera patients were treated by 5 different methods during a period of 5 weeks when the incidence of the disease was high.

The treatment consisted of (1) divided doses of talcum (2) potassium permanganate (3) essential oils (4) bacteriophage and (5) M & B 693 (2-sulphanilyl aminopyridine). These different treatments were given to 90 40 47 44 and 6 patients respectively with percentage mortalities of 18.9 18.5 10.7 4.5 and 10. Thus the mortality was lowest in the group treated with bacteriophage.

The results of cholera therapy were striking and sufficiently encouraging to justify the adoption of bacteriophage as a routine measure in the treatment of cholera. It is suggested that other methods should be compared with the results of bacteriophage therapy. The results of treatment in this experiment are stated to be better than those obtained previously by Pasricha *et al* in 1936. The bacteriophage used was prepared by a somewhat modified method in which the strains used for the propagation of bacteriophage were added in groups at hourly intervals to the seed bacteriophage. The bacteriophage was filtered two hours after the addition of the last batch of strains of cholera vibrio. Pasricha *et al* point out that the importance of this experiment lies in the fact that the different methods of treatment were carried out at the same time during the maximum incidence of the disease in the same ward and amongst people of more or less the same strata of society. Briefly the results obtained by the 5 different methods of treatment described demonstrate that bacteriophage gives the best therapeutic results. Morison and Rice (1934) had somewhat similar results.

Asheshov Khan and Lahri (1936) have reported very favorably upon the bacteriophage treatment of cholera when it is given simultaneously with hypertonic saline. They recommended giving bacteriophage by the mouth in 1 dram doses every 30 minutes undiluted. Two bottles of bacteriophage each containing 50 cc should be given in 16 hours. During the following 24 to 48 hours another 50 cc might be given. They also employed bacteriophage in intravenous inoculation in doses of 5 cc considerably diluted in order to prevent the occurrence of anaphylactic shock.

In regard to individual prophylaxis in India in 1928 in 4 villages 107 received no phage and 68 (63.5 per cent) died 47 received phage and only 5 (7.3 per cent) died. The mode of administration to patients was as follows: 2 cc of culture were added to 10 cc of water and swallowed by the patient, 4 cc in 40 to 50 cc of water were left with the individual and a tablespoonful was to be taken every hour. Next day if the condition was still serious 3 doses of 2 cc were repeated. There was no selection of cases. Those refusing the treatment served as controls. Twenty were so treated and 0 died (85 per cent). Of the 240 who

refused treatment 143 (60 per cent) died. It was believed that the effect depended particularly on the virulence of the phage rather than the amount.

**Bacteriophage in Prophylaxis**—Morison (1932) has added phage in a number of instances to water supplies in Assam and has claimed good results. Two areas were selected, one being kept as a control. In the test district receiving bacteriophage the area remained free from cholera for 5 successive epidemic seasons while in the control area there was one outbreak each season.

Asheshov (1930) believed that there are 3 strains or types of cholera phage which he designated as A, B and C. He was of the opinion that all 3 must be present to be effective against cholera vibrios. Mixtures of all 3 were tried as a prophylactic measure by adding to the water of wells 50 cc. of the phage in the village and town in one district in Behar with the result that there was a marked drop in the incidence of cholera.

In Furi in 1929 in cases arising among the pilgrims the treatment of actual cases in hospitals with bacteriophage was disappointing. Scott (1939) points out that at this time the necessity for the multiplicity of the 3 phage elements had not been recognized. He also points out that to compare the fatalities in epidemics, one in which the phage was used while in another it was not is fallacious because the case mortality varies too widely in different outbreaks for comparison to have any validity. Also to add phage to a water supply before a village is attacked is open to the rejoinder that the infection might not be introduced at all or if it were the type might be mild.

To illustrate an example of the use of bacteriophage in prophylaxis he points out that in 1927 in a village in India of 345 dwellings the water supply consisted of 9 private and 13 public wells. On August 2 there were 6 cases of cholera and 3 deaths and the next day 6 cases and 2 deaths. On August 4 3 cc. of potent bacteriophage were added to 2 wells in the contaminated area. There was only one case subsequently. On another occasion 40 cc. of bacteriophage were passed into the water supply of 10 villages in which cholera had existed for a long time with a high fatality rate. In a day or two the epidemic ceased. He remarks that this seems to be too dramatic to be merely a coincidence.

The use of bacteriophage as a prophylactic has also been employed in Habiganj and Nowgong in 1934. The decisions regarding its value however were apparently uncertain.

Scott drew the conclusion from them that the use of cholera phage when employed alone on a large scale as a preventive must be regarded as still *sub judice* but that as a means of limiting spread of the disease bacteriophage alone is at least as effective as inoculation when the latter is used as a preventive measure after the appearance of the disease.

Rice (1934) and his associates after a careful study of Bacteriophage in the treatment and prevention of cholera concluded that the results established a sufficient probability in favour of a significant effect of the administration of bacteriophage to form a basis of practical policy in the treatment and prevention of cholera in villages.

Buce White (1938) has reported that all strains of *Vibrio cholerae* from Indian sources were found by him to carry a particular bacteriophage, the LL cholera phage, whereas no strains of the vibrios he obtained from the Dutch East Indies was this phage found. It was also not found in the El Tor vibrio. He suggests this may facilitate diagnosis of these strains.

#### TREATMENT

Some authorities have emphasized that in the treatment of cholera one must aim at (1) the destruction and removal of the cholera vibrios

from the body (2) the neutralization of the toxins, (3) the prevention of secondary infections through the damaged mucosa of the intestine and (4) the relief of unfavorable symptoms. However we know today that we cannot by any known treatment accomplish anything of importance in regard to the first three of these measures. What however we can hope to accomplish by direct treatment is (1) to replace the great loss of fluid from the body which occurs so commonly in cholera and at the same time diminish the toxæmia and (2) prevent the occurrence of uræmia.

While it is desirable to discuss the treatment of cholera separately for each of the clinical stages of the disease it should be borne in mind that throughout the course the treatment must above all be symptomatic. It is important that the cholera patient receive treatment from the onset of the infection and everything that is possible should be done to preserve his strength.

Sufficient stress has often not been laid upon the treatment of the first stage of the disease namely the incubative one. During epidemics the people should be advised to seek medical attention upon the appearance of any gastro intestinal disturbance. If the patient comes under observation in the first stage, in which diarrhoea is the most definite and common symptom he should be immediately placed at rest and kept in bed the evacuations being received in a bed pan. He should be undisturbed by unnecessary bathing changing of bed linen etc. It is particularly desirable that he should not be moved. An attempt should be made to check the premonitory looseness of the bowels. No food should be allowed other than rice or barley water. Morphine grain  $\frac{1}{4}$  with atropin (grain  $\frac{1}{150}$ ) hypodermically or chlorodyne minimis 15 by mouth have been recommended and during the first 24 hours are often of service. Beyond this time these drugs should not be administered. It has been asserted that if the diarrhoea is arrested and the intestine set at rest for example by some form of opium a better opportunity is offered for the cholera vibrio to multiply and elaborate its toxin. Actually however such a condition does not seem to result and while opium should not be employed in the later stages of the disease its use is not contraindicated during the incubative stage.

Long experience with the use of castor oil neutral salts, and other purgatives including calomel has demonstrated that treatment with these drugs frequently if not usually exercises an unfavorable influence over the course of the disease. In the human intestine the cholera organism multiplies most rapidly in a fluid medium moreover the action of these purgatives tends to increase the catarrhal condition and impair the resisting power of the mucous membrane of the intestine. Therefore the purgative treatment during this stage cannot be recommended and the indications are to limit peristalsis and to put the intestine at rest. Practically all the intestinal disinfectants that could be tried by the mouth have also been made use of during the premonitory stage but so far without satisfactory result. Either these substances become too dilute

before they reach the organism in the lumen of the intestine or the bacteria have already penetrated too deeply into the glands of the mucosa for the disinfectants to reach them. Formerly calomel in divided doses continued for one or two days was recommended by several authorities.

Rogers previously employed a single dose of chlorodyne followed by astringent remedies such as kino and dilute sulphuric acid. More recently he recommended permanganate of potash. He believed that the permanganate acts by oxidizing the cholera toxins thus destroying or rendering them innocuous. The quantities given of course are too small to destroy the organisms themselves. He advised that the permanganate of potash be powdered finely mixed with kaolin and made up with vaselin into 2 gr (0.2 gm) pills and then coated with melted salol or 1 part of salol with 5 parts of sandarac varnish or with keratin. It is said that these pills dissolve in the small bowel and give off the permanganate slowly without irritating the mucous membrane. In acute cases 2 gr (0.2 gm) may be given every quarter of an hour for the first 2 to 4 hours and then 2 gr (0.2 gm) every half hour until the color of the stool changes to greenish or yellow. As much as 50 to 100 gr (3.25 to 6.5 gm) of permanganate have often been given by him in the course of from 12 to 24 hours. He has also used solutions of permanganate given to the patient to drink but he remarks that the patients sometimes object to the astringent taste of the drug. It has not been determined however that the permanganate given in this way has sufficiently destructive action upon the cholera organism or its toxin in the human intestine to exert any favorable influence on the patient. Long experience has demonstrated that it is better not to administer by the mouth a cathartic that is not essential for the patient and that the best results are to be obtained by bringing about as complete a rest of the intestine as possible.

**Subsidiary Measures**—A suspension of aluminium silicate (kaolin) by the mouth has been particularly recommended by a number of observers from the onset of the cholera symptoms and throughout the course of the disease especially with the idea of preventing the absorption of the cholera toxins from the intestinal tract.

In earlier years favorable results were reported from its use by Stumpf in the Serbian epidemic, Kuhne in the Balkan wars, Walker (93) in India and others. The kaolin powder 10 grm is suspended in 50 cc of water and it is recommended that a glass full of this be sipped every hour or every half hour during the day. Not more than 6 glasses full of 200 gms should be taken in the first 12 hours. On the whole however more recent results of treatment with this drug have not been sufficiently favorable to advocate its use.

Treatment by essential oil has been especially recommended for treatment in India and is still employed by some as one of the standard methods of treatment for the natives in that country. Tomb and his associates who have especially recommended it believe that it has not only the property of reducing the mortality but also has prophylactic value. The following prescription is recommended:

R Sp. aether	30 m
Oil anis	5 m
Oil cajuput	5 m
Oil jun p	5 m
Acid sulph. aromat	15 m

Half a drachm is given in half an ounce of water every quarter of an hour. The average total dose should be 8 drachms.

Tomb has claimed that in 95 per cent of the cases a recovery takes place within 7 hours of the onset and that vomiting, purging and intestinal distress appear to be immediately controlled.

However Chopra (1936) reports that while with this treatment the mortality in some series was reduced to 20.5 per cent in collapse cases

the death rate was still 72 per cent, whereas in cases treated with hypertonic saline solution in collapse cases the death rate was sometimes as low as 20 per cent. He also reports that essential oils mixture is sometimes irritant to the stomach and may produce sudden suppression of urine.

*The sulfonamides* Pasricha and his associates (1939) have treated 44 cases of cholera with M & B 693 (2 sulphanilyl aminopyridine). However, the mortality was apparently the same as with a group of cases treated with essential oils and was not as good as those treated by bacteriophage (p. 638).

Chopra (1941) and his associates have reported upon the treatment of a series of 218 cases of cholera with *sulfaguanidine* and that it had reduced the mortality. In the saline treated cases the mortality was reported as 63.8% and in the cases treated with *sulfaguanidine* only 3.2%. These figures are difficult to understand since the mortality of 63.8% in saline treated cases is far lower than has ever previously been reported in virulent infections. Moreover, Carruthers (1941) has made a careful study of 50 cholera cases in India treated with *sulfanilylguanidine* and compared them with 88 cases that served as controls. He concluded that the treatment of cholera by *sulfaguanidine* was not shown to be of value. The dosage employed was in accordance with that employed by Lyon in bacillary dysentery. The mortality in the control group was 17% and in the *sulfanilylguanidine* group 1.4%.

Wilkinson (1943) reports that in the epidemic in Hong Kong (1938-1941) *sulfaguanidine* was tried but the results were doubtful.

Huang (1944) has reported upon the treatment of 2 patients with Asiatic cholera members of a labor battalion. Only 1 patient died. He states that although he was afraid that the tablets of *sulfaguanidine* would be expelled by vomiting this occurred in only 3 cases. Only 1 patient died.

In the study carried out in 1943 in the Madras Presidency there was a mortality of 36.6 per cent in the 314 treated cases and a mortality of 43.5 per cent in the 356 controls. In the series of 53 cases treated with *sulphasuxidine* the mortality rate was the same as that of the controls. In view of these reports obviously further studies will be necessary to determine the value of this remedy in cholera.

*The premonitory stage* of cholera particularly during epidemics may either be overlooked or be absent or at all events when the patient reaches the hands of the physician this stage has frequently been passed and that of evacuation already begun. During this period of the disease as mentioned purging and vomiting are the most frequent symptoms. Hot fomentations and mustard plasters applied to the abdomen and small pieces of ice given internally may be of some value in checking the vomiting. All medicine by the mouth with the exception sometimes of dilute solutions of cocaine  $\frac{1}{8}$  gr. in 1 teaspoonful of water are of little avail. Alcohol is contra-indicated. Washing out of the stomach has given rise to no good results and even attempts to remove by means of gastric irrigation the cholera poison which it has been claimed by some observers

is excreted by the gastric mucosa have failed. The treatment in this stage therefore resolves itself into an attempt to secure as complete physical and physiological rest for the patient as possible and to conserve the body heat by hot water bottles rather than by too heavy bedclothing. The cramps in the muscles frequently require treatment by massage or brief inhalations of chloroform.

The majority of cases during epidemics come under observation of the physician in the *stage of copious evacuation or of collapse*. The great problem in this stage is to restore or maintain the circulation and if this can be done successfully and the functions of the kidney maintained recovery will usually occur. During the stage of collapse or even when it seems likely to occur opium should never be employed since it may add to the factors which produce anuria later in the disease. During the stage of collapse the pulse the blood pressure and the specific gravity of the blood furnish the most important indications for treatment. If the pulse in the radial artery is present and the blood pressure not too greatly reduced the patient requires little treatment beyond that to conserve the body heat. If on the other hand the pulse loses volume and power and becomes weak and thready stimulants are indicated. Injections of digitalin gr  $\frac{1}{100}$  may be given to stimulate the cardiac action. Hypodermics of 4-6 mgm of adrenalin have also been recommended.

**Intravenous Injections of Saline Solutions** —By far the most valuable treatment of all in the stage of collapse consists in the intravenous injection of saline solution which should be administered in all grave cases. However hypodermic administration of stimulants as indicated above may be necessary in the interval before or during the introduction of the





saline solution. Over half the cholera cases in severe epidemics require intravenous injection for collapse. After the intravenous injection of salt solution even in cases in profound collapse provided a sufficient amount has been introduced the pulse returns at the wrist the face loses its pinched expression the tissues lose their shrunken appearance cyanosis disappears and warmth returns to the skin. The pulse and blood pressure serve as an indicator of the amount to be introduced. When the pulse reaches sufficient volume and the blood pressure has been restored injections should be discontinued. Obviously the saline injection should not be carried to a point where the pulse becomes too bounding and the blood pressure is increased much beyond its normal limit.

In cases of moderate severity 2 liters of saline solution may be injected within 20 to 30 minutes time and it will often be necessary to repeat the injections at intervals of from 6 to 8 hours throughout the day and night. Sellards found that the average patient requires the intravenous injection of 2 liters of fluid every 6 or 8 hours for one or two days.

The question will arise as to whether the saline solution should be given intravenously or subcutaneously. If there is no radial pulse to be distinguished the injection should unquestionably be given intravenously in such instances subcutaneous injections cannot be absorbed in time to be of any value and when the subcutaneous method of injection fails entirely the intravenous method sometimes gives excellent results. The writer has not observed serious results when the solution has been injected judiciously. However a rigor during or following injection is not uncommon. The intravenous injection may be supplemented later by subcutaneous injections and in mild cases copious saline enemata alone may be given frequently.

Perhaps nowhere in medicine do we see the beneficial effects of treatment demonstrated to a greater degree than in the proper employment of intravenous injections of saline solution in the state of collapse in cholera. Many lives are apparently saved by this procedure and the mortality of cholera can undoubtedly be reduced by this method of treatment. However in the great majority of cases after intravenous injections the purging returns often accompanied by the other symptoms of the stage of collapse. Hence constant attention must be paid to the pulse and to the blood pressure in relation to the reintroduction of saline solution. Sometimes it is necessary to continue transfusion at intervals during a period of 48 hours or longer and some patients apparently moribund may require injections of the fluid every 2 or 3 hours during 24 to 36 hours. Rogers lays stress on the specific gravity of the blood as a guide. Blumer however has shown that the estimation of the haemoglobin by an accurate method serves the same purpose. Moreover Sellards (1936) believes that the character of the pulse is usually a sufficient guide and that fluid should be supplied freely before the specific gravity of the blood is increased beyond normal limits.

The other treatment of the stage of collapse consists chiefly in stimulation as indicated by means of full doses of digitalin by conserving the body heat by allaying thirst by sips of iced water and by treatment of the distress and pain. However hypodermic injections of morphine

should only be employed in cases with severe pain after other measures such as the application of heat massage, and even brief inhalations of chloroform have been unsuccessfully tried

Profound cyanosis and apnoea are other symptoms which may occur during the stage of collapse which require speedy and special treatment. These conditions may be brought about partly by the spasm of the pulmonary arteries, the lung refusing to transmit the thickened blood. Frequently only by immediate action can such a case be saved for after coagula have developed in the right heart death is inevitable. The administration of nitrite of amyl or nitroglycerin to overcome the spasm of the pulmonary arteries together with rapid intravenous injection of saline solution is urgently indicated in cases with such symptoms.

**Composition of Solutions for Intravenous Injection**—With the object of preventing the rapid loss of fluid from the body which generally recurs after transfusion with normal sodium chloride solution a number of other solutions have been recommended. There seems to be no doubt that the chloride content of the blood is decreased in nearly all severe cases of cholera but in the first 3 days of the disease according to the results performed in the writer's laboratories in Manila we can scarcely speak of a greater loss in the salts than would correspond to that of the water. Reference has also been made to the fact that in the late stages of the disease the blood again shows an almost normal content of water but the salts are not replaced to the normal amount therefore the blood at this stage has a diminished salt content and is hypotonic. Rogers however has recommended a hypertonic solution for treatment at any time during collapse. He advises for general adoption for either subcutaneous intraperitoneal or intravenous injections the following formula:

Sodium chlorid	gr 120 (8 gm)
Calcium chlorid	gr 4 (0.25 gm)
Potassium chlorid	gr 6 (0.4 gm)
Water (sterilized)	1 pt (568 cc)

During an epidemic of cholera in Manila Sellards and McLau hlin treated two series of cases one with isotonic (0.85 per cent) and the other with hypertonic salt solution. The hypertonic solution contained 1.3 per cent sodium chloride the calcium and potassium salts being the same as in Ringer's solution. The mortality in the cases treated with the isotonic and with the hypertonic solution was practically the same and no advantages whatever were demonstrated for the use of the hypertonic solution.

Strauss believing that hypertonic sodium chloride solutions in large doses do harm to an already damaged epithelium of the kidney has advised the use of an isotonic 4.5 per cent glucose solution for treatment and Kauch a 5 per cent solution of glucose for subcutaneous injection and a 10 per cent one for intravenous injection. Banerjee (1938) has employed 25-50 cc of a 25 per cent solution in some cases.

**Temperature of the Fluid**—Owing to the fact that the temperature may be subnormal in the collapse stage it is important that the saline solution injected should be several degrees above the normal temperature. Nichols and Andrews have recommended that the fluid in the vessel containing the solution should be at a temperature of at least 43 C (109.4 F). They found that the solution after passing through the tube and needle would then have a temperature of not more than 4-6 F above the normal temperature when it enters the vein. Banerjee (1938) employs injections of a temperature as many degrees above the normal temperature as the rectal temperature of the patient is below it. If there is hyperpyrexia the temperature of the injecting fluid should be lower and may be between 80-90 F. The intravenous injection should be given slowly at the rate of not more than 4 oz (120 cc) a minute the flow being slowed down to 1 oz (30 cc) a minute should distress or headache occur.

**Rectal Administration of Saline Solution.**—During the stage of collapse the first important decision to be made in treatment is whether the saline solution shall be

given intravenously subcutaneously or per rectum. Unless the clinical appearance or the blood pressure demand the intravenous injection the solution should be given per rectum. No case should receive an intravenous injection unless the indications are decidedly in favor of such treatment. The indiscriminate use of intravenous injections of saline in cholera is dangerous. Greenwald has recently shown that all sodium salts injected in excess are toxic and that there is produced a sudden and marked disturbance of the relation between sodium ions and other cations. It should also be borne in mind that after intravenous injections the return of the symptoms of evacuation is usual. Even in severe cases where it is necessary to give intravenous injections it is also advisable that injections of fluid per rectum be given. In the stage of evacuation much of the fluid will be rejected but some is usually retained and in mild cases the need of intravenous injections is often avoided. One half liter of the saline or alkaline solution may be given every 2 hours until the collapse stage is passed.

**Other Treatment in the Collapse Stage**—In addition to the above methods of treatment much fluid may be taken into the system by the mouth. It is useless to give large quantities at a time on account of the vomiting but by allowing an ounce or two at a time with short intervals the patient will frequently retain a large amount. When the temperature in the rectum is not below normal ice may be given to suck. Dilute acids both mineral and organic have been recommended from time to time in the treatment of cholera but this method of treatment has been generally given up as being of no advantage. The permanganate treatment has already been discussed.

**Treatment of Anuria and Uraemia**—It has been emphasized that in the stage of collapse suppression of urine often occurs. Every effort must be made to restore the blood pressure to normal. By far the most important symptom requiring treatment in cholera apart from the stage of collapse is that of anuria and the restoration of the urinary excretion is the most important symptom in determining the prognosis after the patient has survived the stage of evacuation.

It is of interest to recall the statistics collected by Rumph and Frankel in relation to this symptom. Of about 700 cases of cholera in which no anuria existed even in the first days of the attack although the urinary secretion was considerably diminished only about 4 per cent died. In 1000 cases in which anuria was observed 57.2 per cent died.

Coffee in small amounts by the mouth if it can be borne by the patient or caffeine with sodium benzoate 5 gr. may be of some slight benefit during the stage of collapse in stimulating the action of the heart and kidneys and digitalis is sometimes indicated. Stimulating diuretics in general however should not be employed in cholera uraemia. The use is of doubtful benefit and they frequently do harm. Cupping sweating and hot packs are not to be recommended for the treatment of the uraemic symptom.

Recently Sellards has emphasized the fact that the relief of uraemia in cholera is intimately connected with the problems concerning the treatment of acidosis. In the study of the urine in this disease he found an almost constant increase in the excretion of ammonia and that cholera patients showed a distinct tolerance to alkalis that is a considerable excess of sodium bicarbonate was required to render the urine alkaline as compared with normal individual. Thus he found that even after relatively enormous injections of bicarbonate of soda (90 gm.) the urine of cholera patients sometimes remains sharply acid while in normal individual is a small amount (3 to 5 gm.) sufficient to change the reaction of the urine from acid to alkaline. More recent investigations demonstrated that this tolerance to bicarbonate is due to an acidosis or more correctly

to a deficit of the body in fixed bases. The acidosis in cholera is obviously not specific but is similar to that observed in nephritis and uraemia from other causes. From the results of the tests of tolerance to bicarbonate in cholera it was demonstrated that acidosis usually made its appearance early in the stage of reaction of the disease and that the degree of acidosis increased rapidly and reached its maximum in those cases showing the most marked evidences of uraemia.

Very satisfactory results were obtained in the relief of this uraemia by treatment with alkalis. Rogers and Shorten later confirmed these observations and demonstrated that a greatly reduced alkalinity of the blood is a constant feature of severe cholera.

Reference has been made to the importance of carefully watching the pulse, the blood pressure or the specific gravity of the blood in connection with the administration of saline solutions and it is also important to observe the reaction of any urine that is passed or that is obtained by catheter in connection with the administration of sodium bicarbonate solution.

For the intravenous injection of alkali Sellards recommended during the stage of collapse a solution composed of 0.5 per cent sodium chloride and 0.5 per cent sodium bicarbonate. Early in the stage of reaction 1.5 per cent of bicarbonate was substituted without the addition of any sodium chloride. If the urine does not become alkaline to litmus after the injection or if the amount of alkali remains small it is recommended that the bicarbonate be increased to 2 per cent. He found the weakly alkaline solution of 0.6 per cent as satisfactory as the neutral saline for the treatment of the stage of collapse. He emphasizes that it is imperative to use bicarbonate and not the normal carbonate and that in sterilizing certain precautions must be taken on account of the ease with which bicarbonate is converted to carbonate by heat. The bicarbonate solutions may be sterilized in an autoclave in an atmosphere of carbon dioxide or they may be sterilized in an open vessel and a stream of sterile carbon dioxide passed through the solution after cooling.

Foster in comparing 2 groups of cases of cholera, one treated with sodium chloride solution and the other with alkaline solution, noted that the most important clinical difference was the absence of uraemia in the group receiving bicarbonate. The only unfavorable results which have been observed from the injection of alkaline solutions in cholera is the appearance sometimes of a moderate and temporary haematuria and mild convulsions. These disturbances however have only very rarely been observed and may have been due to the conversion of sodium bicarbonate to carbonate.

The sodium carbonate may exert a lytic action on the red cells *in vitro* and may cause convulsions but the bicarbonate even in 4 or 5 per cent solutions has no haemolyzing effect.

Greenwald believes that tetany which occurs after large doses of sodium bicarbonate is not due to alkalosis but to the high concentration of sodium salts. He points out that when the convulsions appear after the injection of sodium carbonate or bicarbonate the concentration of sodium in the plasma may be the same as when convulsions appear after the injection of sodium chloride or sulphate. Rogers states that as the use of the alkaline solution produced such a great reduction (70 per cent) in the deaths from suppression of urine while the reduction in the alkalinity of the blood was found to be constant in severe cases of cholera, he recommends first in all cases which are treated by injection 563 cc. of the sodium bicarbonate solution unless the urine is found to have been already rendered alkaline. Sellards believes that the early and persistent use of alkalis has practically eliminated death from uraemia in cholera.

Turnbull (1938) now employs a routine treatment which consists in administering first intravenously sodium bicarbonate 160 gr sodium chloride 60 gr water 1 pint in order to counteract acidosis. This is followed by intravenous hypertonic salt solution. The fluid is stopped when the pulse and blood pressure return to normal and the state of the pulse is the index for repetition of the treatment. Atropine  $\frac{1}{50}$  gr is given twice daily to prevent pulmonary oedema. The series of cases treated numbered 400 and 500 and the deaths 35 to 40.

Banerjee (1939) has called attention to a renal failure type of cholera in which hypochloræmia may be of more importance than simple dehydration. He suggests that the absorption of histamine may in some cases be responsible for the profound fall of blood pressure. Chatterjee (1940) has found that histidine in culture media is transformed by the cholera vibrio into histamine.

Massias (1938) has also emphasized the grave features of cholera due to hypochloræmia and believes that chloropaenia may be a more important symptom than dehydration. In treatment of such cases he injects intravenously 20 cc of a 20 per cent solution of sodium chloride. The dose is repeated 12 hours later if necessary. The mortality was thus lowered to 22 per cent. He suggests that a 10 per cent solution may be an even better concentration than 20 per cent but certainly a 30 per cent concentration is harmful producing tachypnoea and arterial hypertension.

During some outbreaks of cholera the mortality among those receiving saline injections may be higher than among those who are treated differently. Thomas and Ting (1938) have found that unfavorable symptoms following intravenous injections may be due to pyrogenic substances in the distilled water used for the injection. Obviously every precaution must be taken to insure that the saline introduced into the vein is sterile and all precautions to insure this cannot be too carefully carried out. If this is done untoward reactions and rigors are usually avoided.

**Treatment of Stage of Reaction**—After a patient has survived the collapse stage and has entered upon the stage of reaction it must be borne in mind that he is by no means out of danger and also that collapse may recur. The 2 great sources of anxiety are

(1) That the body temperature rises and hyperpyrexia may occur and (2) continued failure of the kidneys to secrete may end in uraemia. The stage of reaction is usually accompanied by some rise in temperature and the intravenous injections may themselves sometimes give rise to a moderate increase in temperature. For the treatment of hyperpyrexia copious enemata of iced saline solution are recommended. Ice should be applied to the head and cold sponging should be employed until the temperature falls. A surface temperature of over 103.5 and a rectal one of over 104 are indications for such treatment. The patient of course should not be surrounded with hot water bottles when the temperature is elevated and indeed these should be used even in the stage of collapse only when the temperature is subnormal. Drugs must not be given or only employed cautiously in the stage of reaction to check the diarrhoea as such treatment seems to lead to an increased absorption of toxins through the damaged intestinal mucous membrane. Opium and lead are particularly dangerous at this stage as they predispose to the condition of uraemia the treatment of which has already been discussed. Should the tongue be coated and the secretion of bile violently interfered with the administration of calomel in small doses may be employed. During the stage of reaction should slight predisposition to uraemia continue alkaline saline solution may be given per rectum by the drop method according to the following formula.

Sodium chloride	14 gm
Sodium carbonate (crystallized)	15 to 30 gm
Water	1000 cc

The temperature of the solution on delivery into the rectum should not be below 65 F in order to favor retention. When the kidneys begin to secrete freely the concentration of the alkali salts may be reduced. If the uraemic symptoms are more urgent than intravenous injection of alkali should again be employed according to the procedure recommended during the later stages of collapse. In cases in which the blood pressure remains persistently low during the stage of reaction pituitrin or adrenalin solution hypodermically are sometimes of benefit.

Naame has claimed particularly favorable results for adrenalin therapy in cholera giving 4 to 6 mg per day subcutaneously for several days together with saline intravenous injections. He considers the cholera toxins in severe cases to have an elective action on the suprarenal capsules. He believes the great tolerance shown by the cholera

patient toward adrenalin is a sign that an active principle which the disease is destroying is being restored to the organism. Manson Bahr recommends pituitary extract  $\frac{1}{2}$ -1 cc injected hypodermically 2-4 times a day. Vitamins such as ascorbic acid or thiamin hydrochloride may be given during convalescence if there is evidence of such vitamin deficiency.

For routine treatment during epidemics the following procedures have been recommended by the Medical Department of the United States Army.

*Restoration of Body Fluids*—This is the most important therapeutic objective and should be promptly and adequately attacked. Fluids should be given liberally by mouth unless contraindicated by vomiting or nausea. It will usually be necessary to supplement oral administration by parenteral injections. This may be accomplished by the intravenous administration of hypertonic saline solution prepared as follows:

Sodium chloride	13.75 grams
Calcium chloride	0.25 gram
Distilled water	1000.00 cc

This aids in replacing salts lost by diarrhoea and assists in retaining fluid in the blood vessels thus maintaining the blood pressure and increasing the excretion of urine. The average cholera patient will require two liters of this solution every 6 to 8 hours for one or two days. The injection should be given slowly and continuously and it may be advisable to tie in a canula because of extreme restlessness of collapsed veins. The pulse and blood pressure should be watched carefully and if there is no suitable response to a given injection it should be repeated within 2 or 3 hours. The intravenous or subcutaneous administration of normal saline may be employed 1000 cc every 4 hours until dehydration is relieved. In giving large amounts of parenteral fluid caution should be taken not to exceed the requirements for normal hydration. If time and equipment permit specific gravity of the blood may be used as a guide to fluid requirements. Rogers suggested this might be determined as follows:

Prepare a series of solutions of glycerin and distilled water of specific gravities 0.002 apart from 1.050 to 1.070 (i.e. 1.050 1.052 1.054 etc). Place small portions (10 to 15 cc) of these solutions in small bottles. Place one drop of blood in each bottle. The specific gravity of the blood is indicated by the bottle in which the drop of blood neither rises to the top nor sinks to the bottom of the solution.

Administer the saline slowly and continuously. Rogers suggested the amount given should depend on the specific gravity of the blood as follows:

- If the sp. gr. is 1.062 give 1000 cc
- If the sp. gr. is 1.063 give 1500 cc
- If the sp. gr. is 1.064 give 2000 cc
- If the sp. gr. is 1.065 give 2500 cc

However, no fixed rule can be established for all cases and the amount administered should depend not only on the specific gravity of the blood but also upon the blood pressure and pulse. Repeat saline injections every 4 hours until specific gravity of blood drops below 1.062. The normal is 1.056 to 1.058. If the patient is dehydrated and equipment for determining specific gravity of the blood is not available administer hypertonic or normal saline using judgment as to amount. Phillips, Van Slyke et al (1943) have devised an improved method using copper sulphate solutions of known accurate specific gravity.

*Treatment of Acidosis and Suppression of Urine*—To combat anuria or marked acidosis use the following solution intravenously:

Sodium chloride	5.75 grams
Sodium bicarbonate	18.25 grams
Distilled water	1000.00 cc

This solution should not be sterilized by boiling or autoclaving as the temperatures reached during those procedures may change the bicarbonate to the caustic carbonate.

*Scudder has employed the method of Barbour and Hamilton.*

The following technique may be employed. Dissolve the sodium chloride (5.75 grams) in the distilled water (1000 c.c.) and sterilize by boiling. Remove from the heater and at once add sodium bicarbonate (18.25 grams) which has been taken directly from the original container and weighed in a sterile vessel. The solution should be cooled to body temperature and used at once. This solution should be prepared and administered with great care and the patient observed carefully for signs of tetany or other manifestations of alkalosis.

**Control of Shock.**—In stage of collapse add 50 grams of glucose to each 1000 c.c. of saline solution administered. Injecting not more than 1000 c.c. per 30 minutes or more than 400 grams of glucose daily. If sugar appears in the urine inulin may be given hypodermically as indicated. Glucose solutions for intravenous injections should be supplemented with 2 mgm. thiamine hydrochloride for each 50 grams of glucose. If normal human serum or plasma is available for intravenous use it may be used as a means of controlling shock, but not as a substitute for other fluids which are essential [serum or plasma have not yet been shown to be of value in cholera].

Keep the patient in bed and apply heat to the abdomen and extremities as long as required. Watch the blood pressure and if below 100 systolic give saline or plasma as indicated above.

**Diet.**—During the acute stages of the disease nothing should be given by mouth with the exception of water or rice or barley water. Too early administration of milk, soups and jellies containing animal albumin is not advisable. Upon resuming food after two or three days farinaceous substances should be given at first. As long as the kidneys are not acting freely an increase in the diet should not be made. Patients should be kept in bed for several days after the acute symptoms have subsided as sudden cardiac failure may occur in patients who sit up before convalescence commences.

**Serum Treatment.**—The serum treatment of cholera has been very unsatisfactory. Owing to the lack of success from the employment of bactericidal sera in the treatment of cholera many attempts were made to prepare antitoxic sera.

In the treatment of a series of cases of cholera in Manila with two sera (prepared by Brown and Denier of the Pasteur Institute) the results of treatment carried out by Denier were as follows:

RESULTS OF SERUM TREATMENT

Injections	No of cases	Cholera parvum not isolated from the stools	Dead	Recovered	Percentage of mortality
Controls	21	3	13	5	72
Serum A antitoxic	16		11	4	75
Serum B antimicrobial	5		2	3	40

From this table it is evident that the cases which received the antitoxic serum were not benefited by it the mortality being even higher than in the ones which received no serum. The number of cases which received the antimicrobial serum is too small to justify decided conclusions although the mortality is much lower.

The effect of treatment with other of these sera prepared with the idea of possessing antitoxic properties was particularly observed in the



epidemic of cholera in Russia. Berthenson of St. Petersburg has reported upon 636 individuals who were treated with various cholera immune sera. Those employed were the sera of Kraus, Salimbeni, Schurapoff and of Kollé, Carrière and Tomarkin. Of the cases treated with serum 322 died or a mortality of 51.2 per cent. Since about one half of those attacked with cholera usually recover with various methods of treatment the results offer no indication of any value for the serum treatment employed as a whole. Other reports show that 133 cases were treated with the serum of Kraus and of Salimbeni in several different hospitals and the favorable effect of the serum as employed in these institutions appeared doubtful according to the reports of Kernig, Ketscher and Jegunoff. A number of other observers have also failed to see any favorable action of the serum of Kraus upon the course of the disease or upon the mortality.

During the epidemic of cholera in the Balkan campaigns cholera serum was extensively employed for treatment but it is difficult to determine its value from the reports that have been made since it was usually employed at the same time with other well recognized measures of efficacy. The serum was obtained from the Pasteur Institute in Paris, from Berne, Vienna and Dresden, no difference in treatment being noted with the various samples. It was generally given intravenously, sometimes in saline solution in doses varying from 10 to 100 cc. The opinions regarding its efficacy were divided among the different Greek physicians. Some believed it to be of value while others saw no good results from its use. In the Salonika Hospital the mortality of a series of very severe cases treated with the serum in 40 to 80 cc. doses according to Savas was 55.7 per cent. Savas however considers that when the serum is given intravenously sufficiently early in the disease and in combination with saline injections it is apparently productive of good results in many cases.

Ghosh (1936) has recently employed anticholera serum prepared with the object of obtaining both endo- and exotoxin. Forty seven cases of cholera were treated in Calcutta the serum being given by the intraperitoneal route. Quantities of from 70-80 cc. of the concentrated serum were given to a selected series of cases in which the blood plasma had a specific gravity of 1.064 or over. The mortality rate in these cases was reported as approximately one half that of the cases treated by other methods. Further trials of this method are desirable.

From a consideration of these observations it will be seen that no one has reported a lower mortality in a large series of cases treated with serum than has been obtained by careful treatment with intravenous injections of saline and alkaline solutions. The average mortality during severe cholera epidemics is usually from 50-60 per cent. In cases carefully treated symptomatically with saline and alkaline injections this mortality may often be reduced to about 20 per cent.

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## Chapter XVIII

### PLAGUE

#### DEFINITION AND SYNONYMS

**Synonyms** —Oriental plague Black death Pest

**Definition** —Plague is an acute febrile infectious disease characterized by inflammation of the lymphatics with the production of buboes septicaemia primary or secondary pneumonia petechial and diffuse haemorrhages and a high mortality The disease is caused by a bacterium of the haemorrhagic septicaemia group *Pasteurella pestis* found during life in the buboes and frequently in the blood At autopsy of fatal cases it is invariably found in the blood and all the organs

Plague is primarily a disease of rats and other rodents in which it exists in both an acute and a chronic form The acute disease in the rat is frequently septicaemic so that when certain species of rat fleas feed on the blood of their host they ingest plague bacilli Especially when the rats die such fleas will attack man and may cause human plague

Bubonic plague is most commonly transmitted to man through the agency of fleas rat or human while primary pneumonic plague is usually transmitted from man to man aerially Primary septicaemic plague is most commonly caused by infection occurring through the mucous membranes with pathological material containing *Pasteurella pestis* The most common way in which plague spreads from one country to another and from one city to another is by means of infected rats usually on board ship but sometimes by railway or other conveyance Occasionally however it may be spread by the importation of either an ambulatory or more severe case of human plague Even with our present quarantine methods which are often very efficient it is practically impossible some times to eliminate entire danger of the importation of plague infection

#### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** —Plague is the most fatal of all epidemic diseases and its tragic history is so full of interest that it has often been referred to from time to time in popular literature One may find very profitable reading regarding the disease from a historical standpoint in the history of the plague in London by Defoe in which however the plague outbreak in Marseilles is especially referred to Pepys's Diary also describes the London epidemic Boccaccio gives an excellent description (published 1348-53) of the plague epidemic which was raging in Florence at the time he wrote and that led to the isolation of the group of young people by whom the stories of the *Decameron* were supposed to be told It is

certain that plague is a disease of great antiquity. In the *Bible* one finds mention of plague as occurring centuries before the Christian era in the land of the Philistines. In the *First Book of Samuel* Chapters 5 and 6 mention is made of the disease having broken out in Canaan during military operations against the Israelites. It is stated that the inhabitants of several of the cities were attacked with emerods and that the pestilence caused a deadly destruction.

In Bethschesesch over 50 000 persons died. It is also recorded that in order that the plague might be stayed the Philistines made propitiatory offerings to the God of Israel of golden images of their tumors and of the mice that marred the land. This appears to be the earliest reference to an epizootic among mice in connection with the disease. Hippocrates 460-370 B C. does not describe plague but he mentions that all fevers complicated with buboes are bad except ephemerals.

Rufus of Ephesus about 100 A D. probably gave the first description of plague which has been preserved. He says the buboes that one calls pestilential are very acute and often cause death. Egypt being the center of trade the Phœnician sailors began to scour the known world and apparently the plague was soon spread the first epidemic known in the world's history probably occurring in the reign of Marcus Aurelius 164 180 A D. the second in Egypt in 542.

Some historians maintain that there is not sufficient satisfactory evidence to determine definitely whether the pestilences of the early years of the Christian era were or were not plague. However if they were not plague it is difficult to conceive what disease they referred to. In any case it is generally agreed that there is no doubt of the nature of the Great Plague of Justinian in the 6th century. It is believed to have started in Egypt and reached Syria and the North Coast of Africa thence spreading over a large part of Europe. It is said to have carried off half the population of the Roman Empire and in Constantinople to have caused the death of 10 000 persons in one day. It was described in Gaul as *lues inguinaria* and persisted in Europe and Asia Minor for over 50 years.

In the Indian *Bhagavata Purana* supposed by many to have been written in the 6th century there is a passage which warns people to desert their houses when rats fall from the roofs and jump about and die presumably from plague.

In the 14th century a new European pandemic began and the most noted and fatal one. The two previous epidemics had come from Africa particularly Egypt. The new one invaded Europe from Asia from Southern India and China by way of the Caspian and Black Seas eventually invading Asia Minor Egypt and Europe. It reached Italy in 1346 and England in 1348. In some localities the disease for the first time in history that we know of assumed the pneumonic form. Putrid inflammation of the lungs was noted with expectoration of blood. In the plague at Avignon for the first 6 to 8 weeks the sick expectorated blood and it was said that to come near them was certain death. No one who was attacked survived of the 62 000 in this city. Afterwards buboes appeared in the groin and axilla and some of the sick recovered. It was called the black death in Germany on account of the petechial spots or tokens on the skin and in Italy it was known as the great mortality. Other historians believe the term black death referred especially to the grievous and lamentable nature of the disease. It has been estimated that one fourth of the population of Europe succumbed to the black death but estimates in certain parts of Europe would indicate a mortality approaching 70 per cent of the inhabitants.

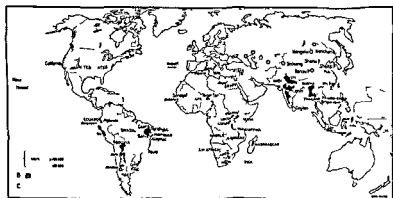
**Quarantine**—It was during this epidemic that the Venetians instituted the first quarantine of infectious areas and quarantine later became a recognized procedure in Europe. The adoption of a period of detention of 40 days probably originated in the medical idea that the 40th day was the last day of ardent diseases this being one of the critical days. The

lazarettos where strangers were held in quarantine appear to have first been established on some island near Venice in 1485

Successive epidemics occurred in Europe in the 15th 16th and 17th centuries and it was in 1630 that the great plague of Milan occurred with its terrible tragedies. The population of this city through fear and cowardice of the pestilence became completely demoralized and plague stricken houses were burned to the ground and persons suspected of spreading the plague by smearing its virus about were put to death with great torture



1917-1935



1936-1939

A.—Active foci of plague

B.—Active foci of plague with a high incidence

C.—Imported foci of plague principally from the East

FIG 154—Geographical distribution of plague (Epidemiological Intelligence of the League of Nations)

See vice

In 1665 occurred the Great Plague of London during which year it was estimated that approximately 60 000 out of a population of 40 000 died. It was thought that this epidemic was introduced from the Levant by way of Holland. Defoe has written a vivid account of the plague of London in 1665 in his book called *A Journal of a Plague Year* which must not be regarded as an accurate description of this outbreak since he

was born in 1659 and was only 6 years old at the time of the London epidemic. The book was published in 1722 two years after the Marseilles outbreak of plague. It is known that he carefully collected material for a diary of the Marseilles outbreak. There was much plague in Europe in the 18th century but it would seem to have completely disappeared by 1841 and only to have returned with the pandemic which began in 1894.

*The Last Pandemic*—The plague epidemic with which all important parts of the world became concerned is supposed to have originated in China, in the province of Yunan on the Tibetan border reaching Canton in 1894 Calcutta and Bombay in 1896. From India it spread to Singapore the Philippine Islands Arabia Persia, Turkey, Egypt and West Africa and later to Russia and through parts of Europe and to the coast of North and South America Central America the West Indies and Mexico and thence to the North American gulf coast. Nearly every country in the world became affected. In 1900 plague appeared in San Francisco. Later the ground squirrels were found to be infected with the disease over about one fourth of the state. In 1907 Seattle became infected and plague rats were found there during the next 10 years.

During more recent years human outbreaks have been observed in the United States not only in California but also in Louisiana Texas and Florida as well as in Mexico and practically all of the Central and South American republics. The disease was introduced into the west coast at Peru from India in 1903 and spread from there to Ecuador. It has also been present in Alexandria Egypt in eastern western and southern Africa and Madagascar. In Asia it has prevailed particularly in India Japan and China the Straits Settlements Turkey and in the large islands such as Java the Philippine Islands and Hawaii and in Australia. In Europe practically all of the Mediterranean seaports became infected as well as a number of the larger ports of England France and Spain. In 1921 60 cases of the disease were reported in the interior of France at Paris and in 1923 14 additional cases at Saint Ouen (a suburb of Paris). In 1921 an isolated case of the disease was reported in the city of Dublin.

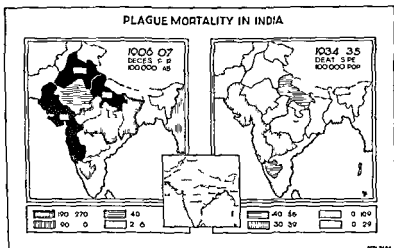
*India* still constitutes one of the great endemic centers of Plague and during the past 20 years there have been approximately 10 000 000 deaths from the disease. Over a million deaths a year have occurred at times. In 1904-5 there were 1 315 000 deaths and in 1906-7, 1 287 000. The tendency for the past 20 years, however has been toward a greatly diminished prevalence of the disease in India. In 1923 there were not over 250 000 deaths and in 1934-35 only about 52 000.

In *Netherlands India* plague was imported in 1911 into a port of eastern Java. The disease did not take root in the low coastal regions but became endemic in the inland mountain areas. Murine and human endemic infection progressed slowly westwards while the areas first infected in the east were gradually freed from rats and plague by the systematic reconstruction of thousands of villages. From 1911 to 1937 the disease caused no fewer than 211 000 deaths.

In *China* according to Wu Lien Teh Chun and Pollitzer (1937) the chief endemic foci of plague have been in the interior of Fukien Province in the south in the upper valley of the Yellow River (Shansi and Kansu) in the west and at Tungshao Manchuria on the north. Williams (1941) points out that though plague had not been present in southwest China for 40 years in 1938 an outbreak occurred just over the border in Burma the epidemic beginning at Mamhkam 50 miles away in Burma. It was stated that it was quickly controlled through wholesale inoculation. A small outbreak principally an epizootic among rats occurred at Hsenwi 75 miles west in Burma during 1939 when an anti rat campaign appears to have controlled this one. Williams

observed in 1940 the presence of plague again in Bahmo at the head of the navigation of the Irrawaddy River but it did not spread into China. During the present year 1941 a new outbreak has occurred in Chekiang Province. In 1942 plague appeared in 11 different localities in China.

## PLAGUE MORTALITY IN INDIA



Deaths per 100,000 population in 1906-07 and 1934-35

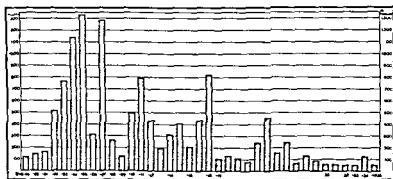


FIG. 155.—Plague mortality in India (Epidemiological Intelligence Service of the League of Nations)

In 1910-11 the disease in Manchuria for the second time in history assumed an epidemic of great proportions of the primary pneumonic form and there were over 60,000 deaths. Another smaller epidemic of this primary pneumonic form occurred in Middle China in 1917-18 and in Manchuria in 1920-21 with 10,000 deaths. Still smaller outbreaks of pneumonic plague in connection with cases of bubonic plague have occurred in California in 1919 and in 1924 and in the Ural region of Russia in 1923-24. In Egypt 120 fatal cases of secondary pneumonic plague were reported during 1923.



During the year 1923 no continent was entirely free from bubonic plague and hence there was always the possibility that cases of this disease might appear in any of the large seaport cities of the world and that even new endemic foci might be established by imported infection. Such an endemic focus of the disease had already been established in the United States in California by 1908.

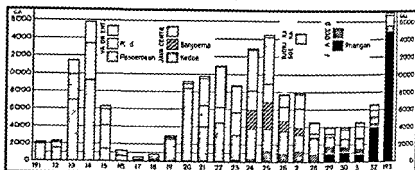


Fig. 150—Course of plague mortality in Java from 1911 to 1931.

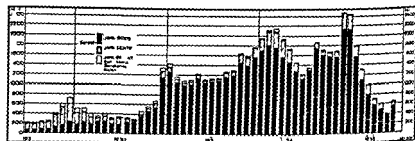


Fig. 151—Course of plague mortality by weeks in Java from 1911 to 1931.

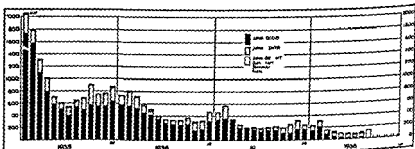


FIG. 150—Course of plague in Java (Epidemiological Intelligence Service of the League of Nations).

**Geographical Distribution**—During the past 10 years plague has continued to decline in India. The League of Nations Health Organization 1938 points out that the mortality has fallen from 121,242 in 1928 to under 7000 in 1938. This mortality is decidedly localized for more than half of it occurred in the United Provinces. It has been suggested that this reduction may be due to the establishment of some degree of immunity in the rat population and it has been said to be roughly pro-

portionate to the prevalence of plague in the areas from which the test rats were taken. Also the disease is essentially bubonic in nature the pneumonic form being very rare. Moreover sylvatic plague is not a problem in India. Field rats other than the domestic species do not exist in large numbers and rural plague is secondary to the house epizootics among *Rattus rattus*. It is somewhat strange that in Ceylon plague has not been a serious problem. Thus in the years from 1929 to 1935 there have been reported not more than 75 cases annually in Colombo.

In Netherlands India as in British India plague has also recently shown a steady fall particularly in Java where the marked decline in prevalence has coincided with the use of mass inoculation by Otten's living vaccine. Rosier (1937) in his report on plague in Java states that the plague reached its height in 1934 with 23 267 cases and 23 239 deaths. It was largely bubonic although pneumonia sometimes complicated it and there were 1506 cases of primary pneumonia scattered among the bubonic cases.

In 1935 the anti plague inoculation was adopted on a large scale and during the year 2 363 642 inoculations were carried out. Such vaccination has been continued with excellent results. In 1937 there were only 6227 cases with 6187 deaths while in 1938 the report gives only 3834 cases with 3814 deaths. Rosier emphasizes that the steady decline is to be attributed in the first place to the use of Otten's avirulent living vaccine. More than 6 000 000 inoculations have been made in 4 years since 1935.

In Africa in earlier years plague was limited to the ports where it was introduced as Mombassa Delagoa Bay Capetown Benguela etc. In later years rats transported sometimes by railway transmitted the infection to some of the wild rodents in the interior. Worthington (1938) reports the disease has now become firmly established the main centers being in South Africa especially Angola East Africa especially Uganda and to a lesser extent certain areas in West Africa. On the whole during the last decade there has been considerable increase in the cases of plague in East Africa.

In Uganda the cases have varied annually from about 100-500 in different years. The mortality however has been unusually high varying from 85 to 97 per cent. Usually Kenya has suffered to a lesser extent but during 1941 to 1942 some thousand cases occurred. Plum (1942) reports that in Nairobi in 12 months 547 patients with Bubonic plague were admitted to the two civil hospitals three of which 354 died. Roberts (1939) who has studied rodent plague in Kenya for a number of years has found no evidence of the infection in wild rodents. Tanganyika has been relatively free from plague for the past several years. In 1934 no cases were reported and in 1935 only one. In Morocco in the vicinity of Casablanca 2200 cases were reported in 1941 and 362 in 1942. An outbreak of the disease started in Suez in November 1943 and up to the 5th of January 1944 a total of 57 cases had been reported the mortality being 71 due to the occurrence of some cases of primary pneumonic plague. In March 1944 the epidemic was still continuing.

In South Africa Thornton (1936) states that the striped mouse (*Rhodonys pumilus*) in the bush has gradually become infected from the domestic rodents. Fourie (1938) reports that infection in 2 groups of veldt rodents is now present all over the South African Union and that the mice are especially concerned in its spread.

West Africa at the present time suffers little from plague. There was a small outbreak in Nigeria in 1929 but none there since 1935. The Gold Coast Gambia and Sierra Leone have also been free. Howler Sorel (1937) reports that in Senegal and Dakar for the preceding several years the extent has been between 1000 and 2000 yearly with about 10 per cent primary pneumonic cases. In Dakar man is infected especially through the intermediary of a domestic rodent (*Mastomys coucha*).

Madagascar became infected in 1898 after a rice steamer from India arrived. The disease was formerly coastal but since 1921 it has become endemic on the high plateaus.

All 3 types of the disease occur but the pneumonic is unusually common in the cooler season. Plague in Madagascar differs from that in South Africa in that wild rodents have not been found to be of importance in transmission. Achard in a recent survey found 1363 cases of which 663 were bubonic 442 pulmonary and 258 septicaemic.

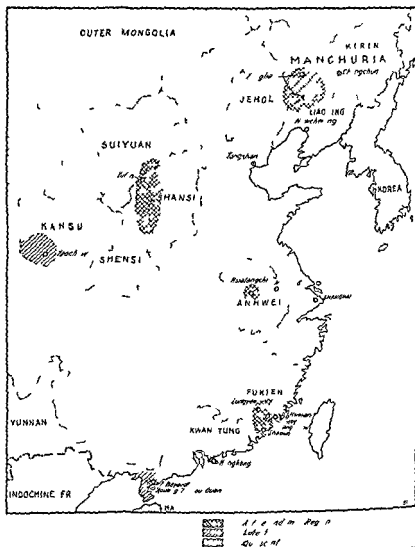


FIG. 157.—Plague 1937 (Epidemiological Intelligence Service of the League of Nations)

The mortality was very high 88 per cent of the bubonic 100 per cent in the pulmonary and septicaemic case. Vogel and Riou (1939) point out that the presence of the disease in Madagascar has been greatly decreased since 1936 which is believed to be due to a continued and extensive campaign since that time of prophylactic vaccination with the living avirulent culture 77.5 per cent of the inhabitants having been inoculated. In 1941 285 cases were reported.

In *South America* plague which was especially prevalent in Brazil in Sao Paulo and Rio de Janeiro from 1900 to 1913 has gradually declined. The decline was first especially due to the gradual improvement in the sanitary conditions and the building of these two modern cities. Plague afterward became sporadic and with the continued improvement in

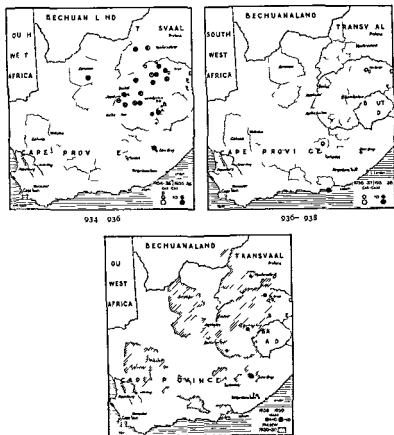
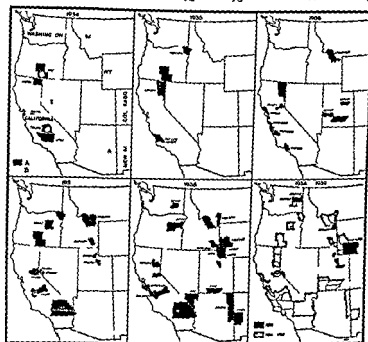


FIG. 58.—Plague—geographical distribution in South Africa (Epidemiological Intelligence Service of the League of Nations)

dwelling houses and the campaigns of rat destruction human and rat plague is now said by Fontenelle (1939) to have completely disappeared in these cities. However Barreto (1938) says the disease is still endemic in the northeastern part of Brazil where in the factories there are plentiful supplies of food for rats as corn cotton and mandioca. From 1934 to 1939 there were 1542 cases in these regions. The plague however is relatively benign and the mortality has usually varied from 35 to 43 per

cent Moll and O'Leary (1940-1941) have published a most comprehensive and authoritative report upon the subject of 'The History of Plague in the Americas' to which the reader is referred for complete details.

In Argentina the disease was first noted in 1900 Alfaro (1937) points out that plague is now very rare though there was an outbreak in the Pampas in 1934 with scattered cases during 10 weeks. There were only 31 cases reported in the entire country in 1936 and Buenos Aires did not record a single case from 1931 to 1936. However Sussini (1938)



A—C. The geographical distribution of sylvatic plague in the United States of America, 1934-1938.

B—G. Geographical distribution of human cases.

FIG. 159.—Spread of sylvatic plague in the United States of America, 1934-1938 (Epidemiological Intelligence Service of the League of Nations).

states that in the Argentine sylvatic plague has assumed some importance because of its tendency to appear in epizootic form and to spread widely over the country. The epizootic makes its appearance in winter, fades out in spring, and reappears the following winter. This engenders at least a small amount of human infection and the appearance from time to time of cases of pneumonic plague. In addition to other wild rodents a species of hare has been found infected. In Tucuman the disease was epidemic and epizootic in 1940 and in Cordova there were 51 cases in 1941.

In Ecuador Murdock (1939) has reported an outbreak of pneumonic plague which lasted only a few weeks but gave rise to 15 deaths among the 16 affected. After the first case was admitted to the hospital rapid contact with infection followed among the nursing sisters, attendants and doctors.

In 1938 a small outbreak of about 100 cases occurred in Bolivia and in 1939 a few more cases in that country and in Peru. In Pernambuco (1941) 102 cases occurred. During 1941-1942 plague was present in Argentina, Brazil, Ecuador, Peru, Bolivia and Chile. In Hawaii in 1943 there were 7 cases and 5 deaths and in New Caledonia 2 cases and 1 death.

In the United States California first became infected in 1900, the infection being brought from Hong Kong. Cases were at first of the bubonic type and mostly in the Chinese colony. In 1907 it spread more widely over the state and in 1908 the infection was found to be present in ground squirrels. During the next 10 years sporadic cases were observed in 6 counties but only 11 human cases occurred. However in 1919 a small outbreak of pneumonic plague was reported in Oakland, 13 cases in all and other sporadic cases were met with during the next 5 years. In 1924 32 cases of pneumonic plague occurred in Los Angeles, 30 of the patients dying. In addition there were 5 cases of bubonic plague, 3 fatal. During the next 10 years only 6 sporadic cases of the disease were reported.

Cumming (1937) reports that there were but 5 cases of human plague in the United States during that year: 3 in California, 1 in Utah and 1 in Nevada, with 105 infections with plague in rodents found in these areas. No human cases occurred in Hawaii but 103 plague infected rodents were found there.

Parran (1937) pointed out that plague infection in rodents is known to exist in 7 western states but had not been discovered east of Wyoming nor south of Utah except in California. By 1939 the infection was found to be present in 10 states in all. The most northern point in the United States discovered was about 150 miles from the Canadian border. It had not then been found east of the Rocky Mountains. Ground squirrels infected then numbered 7 species, especially *Citellus beecheyi*; but the tree squirrel, chipmunk, marmot and prairie dog are all rodents in which plague infection has been found. By 1942 Nevada, Utah and North Dakota were shown to contain infected rodents, North Dakota being the most Eastern state. During the past 40 years some fifty cases of plague have occurred in the United States apparently of sylvatic origin.

In 1941 there were but two fatal cases of plague in human beings reported in the United States, both in Siskiyou County, California. These cases occurred in 2 boys which however lived some 50 miles apart. There has been but one human case in the United States in 1942. Meyer, who has made a most complete study of the entire subject of sylvatic plague, points out that the history of plague in San Francisco and California is one of ebb and flow but never of final disappearance. The importance of sylvatic plague is emphasized as a latent infection among ground squirrels and in his opinion North America from now on will remain a permanent plague focus. He has also shown that at least 15 species of ground squirrels of the Genus *Citellus* are infected and that at least 10 of the Western states are known to harbor infected rodents.

In earlier years the important foci of endemicity of plague which were especially recognized were India, Tibet and Yunnan and Mesopotamia, Uganda in Africa and the Transbaikalian region of Siberia were regarded as less important centers. From what has been said it is obvious that at the present time a number of other endemic centers of rodent plague have developed and are now present in different parts of the world.

#### ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—*B. pestis* (Yersin, Kitasato, 1894) *Pasteurella pestis*. This organism is a member of the group of bacteria which cause the haemorrhagic septicaemias (Pasteurelloses) of various animals. Plague is primarily an epizootic disease of rats but in some localities ground squirrels and other rodents have been shown to be the source of human infection.

It would seem to be impossible for any bacteriologist to fail to see the plague bacillus in a microscopical preparation made from a plague bubo for the organisms are very numerous and frequently in pure culture.

Nevertheless it was not until 1894 in Hong Kong that the bacillus of plague was first isolated and described by Yersin from a plague bubo.

It is true that Kitasato reported a bacillus which he had isolated from the blood of a plague patient on July 7, 1894 (Yersin's report was made July 30, 1894). Kitasato's bacillus was motile, Gram-positive, coagulated milk and gave a turbidity in bouillon.

characteristics which were quite different from those of the organism reported by Yersin—the true plague bacillus

Morphologically *Bacillus pestis* is a bipolar staining organism showing under different conditions considerable polymorphism. In general three forms may be recognized—short rounded or oval forms often appearing as diplococci longer rods and large

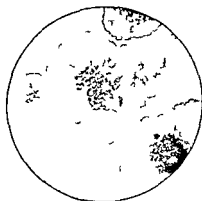


FIG 160—Colonies of plague bacilli 48 hours old (Kolle and Wassermann)

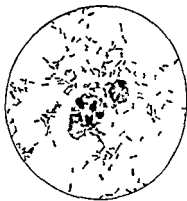


FIG 161—Pest bacilli from spleen of rat (Kolle and Wassermann)

oval pear shaped or club shaped involution forms. The length of the organism varies from 1.5 to 1.75 microns and it is about 0.5 micron in width. For demonstrating the bipolar stain one of the Romanowsky dyes is recommended or carbolfuchsin with subsequent decolorization with 36 per cent acetic acid. It is preferable to fix films by absolute methyl alcohol rather than by heat. The organism is decolorized by Gram's



FIG 162—Plague bacillus involution forms produced by growing on 3% salt agar (Kolle and Wassermann)

stain is non motile and does not form spores. Capsules can often be demonstrated. According to Schutze (1932) the gelatinous capsule does not develop in cultures grown at 20°C. In the early stages of the disease the material obtained from the buboes usually shows the oval bipolar staining microorganism. However after the bubo has suppurated longer rods and involution forms are not infrequent and the typical bipolar staining organisms may not be observed.

**Cultural Characteristics**—Unlike most pathogenic bacteria the plague bacillus grows equally well at about 30°C as at 37°C, a property that can be sometimes utilized in isolating the organism from pus from discharging buboes or other decomposing pathological material which contains *Bacillus coli* or other bacteria which overgrow the plague bacilli at 37°C but would not develop as rapidly at the lower temperature. Slightly

alkaline agar (pH 7.2) is the best medium. After 18 to 24 hours the colonies appear as small dewdrops. Later they assume a whitish gray appearance. Under the microscope there is a dark granular center surrounded by a zone of much lighter growth with greater transparency. A very striking feature in the same culture may be the occurrence of two very differently appearing colonies. It sometimes leads one to think that the culture is impure. The second type of colony is quite different from the dew drop like colonies. The diameter is several times as great and the colonies are thicker

and more opaque. These are sometimes known as cannibal or giant colonies. Morphologically they sometimes contain more involution forms or more of the longer rod like organisms. The use of salt agar 2.5 to 3.5 per cent is of service in demonstrating the involution forms of the plague bacillus which are of some significance from a diagnostic point of view as they often occur more quickly and with greater certainty with *Bacillus pestis* than in the case of other organisms. These involution forms may take a coccoid rod-shaped or irregular sausage shaped form ranging in size from 3 to 10 microns in length. The stalactite growth in bouillon is often quite characteristic. In bouillon cultures there is a cloudiness and then a deposit forms. Around the edge of the vessel a whitish ring or growth appears and this finally spreads over the whole surface as a fine membrane. If a layer of oil (cocoanut for example) is placed on the surface of the bouillon the growth becomes abundant at the surface and the bacilli grow downward in the form of stalactities. The organism is found often in long chains in the liquid medium. The growth in other media is not so important except in differential diagnosis. The plague bacillus acidifies glucose maltose mannite and salicin but produces no gas. Lactose and cane sugar remain unchanged. Indol is not produced and milk is not coagulated. Litmus milk is acidified slightly or remains unchanged.

Colonial variants have been described including smooth compact small fringe large irregular and sunflower types but the exact form of colony produced seems to be so influenced by environmental factors that Topley (1936) believes it is probably better not to refer to these types by the terms rough and smooth. Otten (1936) also refers to the fact that the cultural behavior of *Bacillus pestis* may depend on the nature of the culture medium as the peptone percentage the degree of humidity the pH and many other circumstances unknown. He does not believe that the degree of virulence can be accounted for by the morphology of the colony or as to whether it is smooth or rough.

Bessenova and Ienskaja (1931) found that among 150 plague strains there were a few giving a diffuse turbidity when grown in broth. When seeded on agar medium one of them showed a form of colony distinctly different from the normal type which however was also present on the same plate. The normal type was of the same virulence as the original strain whereas the variant with its atypical growth appeared to be avirulent. In their opinion the atypical variant must be considered as the smooth type. On the other hand Pine found that his rough type of *P. pestis* was avirulent and apparently possessed no immunizing value and it appears illogical to designate the normal virulent type as a rough variant which according to Otten has nothing in common with it.

Schultze (1931) identified two antigenic constituents in plague bacilli a somatic antigen and one occurring in the gelatinous capsule which is only present in cultures grown at 37°C. The former antigen is heat stable and the latter heat labile at 60°C.

*Resistance of the plague bacillus outside of the body* is slight. The organism is completely killed by thorough drying in the sunlight. In cadavers and in the putrid spleen it has been found alive after 4 days. In buried bodies according to other experiments it has been isolated at periods varying from 3 to 30 days after burial depending considerably on the temperature. Ordinarily the plague bacillus does not live in putrid organs more than a week. In Manchuria where the corpses were frozen it was isolated from some of them three months after death. On cow dung floors in India it will remain alive for 48 hours and in grain and meal for 13 days if sufficiently moist. Esley (1930) found that it may retain its virulence for as long as 4 weeks in dried flea faeces. In dust the organism dies out rapidly so it is not likely to be transmitted in currents of air except when frozen. Droplets of sputum in cold weather become an important means of transmission. The organism will remain virulent for a long time in frozen sputum. A temperature of 65°C for one hour



will destroy all the plague organisms. Usually the plague bacillus may be killed at considerably lower temperatures. (In bouillon cultures at  $55^{\circ}\text{C}$ ) It is also destroyed by the ordinary disinfectants as 0.5 phenol after 15 minutes.

**Pathogenicity**—Infection may be produced in susceptible animals (as guinea pigs, rats and monkeys) through slight abrasions of the skin by instillation upon the conjunctiva by cutaneous, subcutaneous or intra



FIG. 163.—Guinea pig plague infection.

peritoneal inoculation by inhalation or by ingestion of cultures. The guinea pig is the most susceptible of all animals to plague infection. Barber showed that by the inoculation of a single virulent plague bacillus a fatal infection could be produced.

However, Bokhey (1939) reported the white mouse (Haffkine Institute inbred strain) as the most susceptible animal, as few as 10 organisms perhaps even less producing fatal infection. Otten (1933) agreeing with most other bacteriologists regards the guinea pig as most susceptible. After this animal he placed the wild rat, the white mouse and the white rat in their order of susceptibility. However, it is well recognized that wild rats greatly vary in their susceptibility to plague infection following experimental inoculation.

Guinea pigs inoculated with cultures of the plague bacillus or with material from autopsies containing this organism usually die in 3 to 5 days. At the autopsy there is (1) marked subcutaneous congestion, oedema and haemorrhage about the point of the inoculation; (2) buboes in one inguinal region and often in both; (3) numerous necrotic and yellowish white foci in the spleen and sometimes in the liver; (4) haemorrhages in the lungs and sometimes in the heart muscle and frequently elsewhere in the body. The typical bipolar staining plague organism can be demonstrated in the microscopical preparations made from the buboes and all the other organs as well as in cultures made from these organs.

In rats which have died of natural acute plague there is usually subcutaneous congestion and oedema and subcutaneous haemorrhages are present. The lymphatic glands, especially the cervical ones, usually show congestion, haemorrhagic necrosis and perilar infiltration. Buboes occur in the cervical or inguinal gland in 75 to 85 per cent of the cases and are most important evidence of the disease. In about 5 per cent the bubo is cervical, in 5 per cent axillary and in 70 per cent inguinal. Another important lesion is the granular liver which is present in about half of the cases. It may have a yellow mottled appearance liberally sprinkled with discrete yellowish white granules about the size of a pinhead. The spleen may be large and injected and granular in appearance (in less than 5 per cent). In the thorax the pleural cavity usually contains fluid (in some 60 per cent) and there are haemorrhages of the lungs and heart. Smears from the spleen and affected glands show the bacilli in great numbers. However, in subacute or chronic infections the bacilli are scanty and their distribution is not constant. For this reason the British Commission believed that the naked eye examination was more important for diagnosis than the bacteriological one alone. However, it is impossible to differentiate plague bacillus infections in guinea pigs and rats from *Bacillus tularensis* (*P. tularensis*) infection by the naked eye. By the bacteriological examination *Bacillus tularensis* can be easily distinguished from the plague bacillus ( ) because it is much smaller (0.3 to 0.7  $\mu$  in length and 0.2 to 0.3  $\mu$  in width) (2) It is decolorized by Gram's stain but it does not grow in ordinary agar or the usual bacteriological media as does *P. pestis*. However, it can be cultivated upon egg yolk and upon cystine agar. (3) The histopathology of *Bacillus tularensis* infection is also quite different and characteristic from that observed in plague.

Wherry first reported 2 cases of ulcerative conjunctivitis with lymphadenitis of cervical glands, fever and marked prostration due to infection with *Bacillus tularensis* (*Pasteurella tularensis*) occurring in persons who had handled rabbits which had died of this plague-like infection. The organism was first noted by McCoy in squirrels in California. The symptoms and macroscopic lesions in these animals are almost identical with those of plague. Guinea pigs succumb after the cutaneous inoculation of material and also show lesions markedly resembling plague. As will be noted in the chapter on tularaemia, the disease has a wide geographical distribution in the United States.

The most conclusive evidence of the presence of *P. pestis* in any pathological material may be obtained when such material is rubbed on the freshly shaven, scarified skin of the abdomen of the guinea pig, the animal subsequently dying of plague infection. However, *P. tularensis* will also pass through intact shaven skin and it produces gross lesions in the guinea pig almost identical to those of plague. Other organisms, however, which might infect through intact skin produce lesions unlike those of plague. As a practical point it may be stated that cases showing a profusion of oval, bipolar staining bacilli in smears from glands or sputum

will destroy all the plague organisms. Usually the plague bacillus may be killed at considerably lower temperatures. (In bouillon cultures at  $55^{\circ}\text{C}$ .) It is also destroyed by the ordinary disinfectants as 0.5 phenol after 15 minutes.

**Pathogenicity**—Infection may be produced in susceptible animals (as guinea pigs, rats and monkeys) through slight abrasions of the skin by instillation upon the conjunctiva, by cutaneous, subcutaneous or intra-



FIG. 163.—Guinea pig plague infection.

peritoneal inoculation, by inhalation or by ingestion of cultures. The guinea pig is the most susceptible of all animals to plague infection. Barber showed that by the inoculation of a single virulent plague bacillus a fatal infection could be produced.

However, Sokhey (1939) reported the white mouse (Haffkine Institute inbred strain) as the most susceptible animal, as few as 10 organisms, perhaps even less, producing fatal infection. Otten (1933), agreeing with most other bacteriologists, regards the guinea pig as most susceptible. After this animal he placed the wild rat, the white mouse, and the white rat in their order of susceptibility. However, it is well recognized that wild rats greatly vary in their susceptibility to plague infection following experimental inoculation.

cases of bubonic plague secondary involvement of the lungs may also occur the lesions being of a metastatic character

Epidemics of plague are usually bubonic in character and in such epidemics there are always a small number of primary septicaemic cases as well as some of secondary plague pneumonia. However a few severe epidemics have been of the primary pneumonic variety. These severe outbreaks have occurred particularly during colder weather and among people who have lived under greatly overcrowded and other unsanitary conditions. Isolated cases of secondary plague pneumonia which occur during large epidemics of bubonic plague are not so liable to give rise to large epidemics as are cases of primary pneumonic plague. Thus among the 519 cases of bubonic plague reported in Egypt in 1923 there were only 120 fatal cases of secondary plague pneumonia and while in that country from 1899 to 1913 there were altogether 83 pure outbreaks of pneumonic plague the number of cases in each was small. In fact in 16 per cent of these instances only one case of pneumonic plague was found. In the bubonic plague outbreak in California in 1919 there were 13 cases of pneumonic plague developing from a case of secondary plague pneumonia and in 1924 there were 30 deaths from pneumonic plague. In the Ural region of Russia with colder weather in the winter of 193-24 there were 9 cases of the pneumonic type and at the same time in the neighboring Kirghiz Republic 14 cases.

While both bubonic plague and pneumonic plague are caused by the same micro-organism *B. C. H. pestis* the portal of entry of the two infections is entirely distinct and pneumonic plague is clinically and epidemiologically a different disease from the bubonic form.

Meyer (1942) in a most interesting article entitled "The Known and Unknown in Plague" calls attention to the fact. The examination of approximately 80 strains of *P. pestis* isolated in California have placed them into the glycerine negative group. They are unable to ferment glycerine and are designated as Beta types (Kuraichi) and they belong according to Berlin and Borzenkov to the Oceanic Race. It is the belief of the last mentioned investigators who have examined and compared plague strains from the continents of the world that these races or types are exclusively encountered in the endemic belt of islands and peninsulas of the tropics (Indo-China, Java, Japan, Ceylon, Arabia, Madagascar and Philippines). They differ from the Continental Race or Alpha type which ferment glycerine and are found in an endemic belt throughout the Central Asiatic Plateau, Mongolia and Manchuria. These facts have been used by Kuraichi to speculate on the origin of plague in California. Since the pandemic starting from Hongkong in 1894 was associated with the Beta or Oceanic Race he believes that ample evidence is available to consider the North American enzootic foci to be the descendants of this race. Originally it was brought to San Francisco and spread from rats to the squirrels. Meyer points out that he overlooks an important detail in that the South African sylvatic plague focus is likewise attributed to the pandemic dispersion at the turn of the century. Although the 23 plague strains studied by Pirie are predominantly glycerine negative at least 4 strains of the Continental Race have been discovered in the Cape Colony. Perhaps in time the examination of plague cultures from diseased rodents or ectoparasites East of the Rocky Mountains might yield such races. Until such tests have been made it is probably inadvisable to use biochemical characteristics to bolster up one or the other theory concerning the origin of plague on the North American Continent.

**Transmission.**—In bubonic plague the infection is usually acquired through the skin. Epidemics of bubonic plague are associated with rodent infection. Man acquires the infection usually secondarily from the rat, the rat flea transmitting the plague bacillus.

#### SIPHONAPTERA

The fleas are classified in the order Siphonaptera. They are laterally flattened markedly chitinated wingless insects which undergo a complete metamorphosis.

This order is divided by Dalla Torre into two sub-orders—(1) the Fracticipita which contains the family Hystriopsyllidae of which the genus *Leptopsylla* is of medical interest and (2) the Integricipita containing the following families and genera of medical interest: Pulicidae—genera *Pulex* and *Xenopsylla*; Archaeopsyllidae—genus *Ceratophyllus*; Dolichopsyllidae—genera *Ceratophyllus* and *Hoplopsyllus*; Tungidae—genus *Tunga*.

and with clinical manifestations of plague are not likely to be other than plague bacilli. Still to be conservative one should always inoculate animals cutaneously or subcutaneously.

*Other Organisms of the Haemorrhagic Septicemia Group*—In making rat surveys for the detection of plague other spontaneous infections may be sometimes encountered in which the causative organisms may show bipolar staining be Gram negative and more or less similar in their cultural appearance to *Past pestis*. Of these *Past Pseudotuberculosis rodentium* (*Corynebacterium pseudotuberculosis* Bergey) is related to *Past pestis* antigenically. According to Schutze the somatic antigen is common to both species.

*Past pseudotuberculosis rodentium* may give rise to a fatal septicaemia in rats and guinea pigs often accompanied by the formation of nodules in the spleen and liver and sometimes the lungs. As a rule the greyish white nodules are larger than those of plague up to 3 mm in diameter and may stand out more from the surface. In these lesions large numbers of short coccoid or ovoid bipolar staining Gram negative bacilli occur. On injection of this organism into guinea pigs rats or mice infection usually proves fatal only after 1-3 weeks.

One aid in differentiation is by the injection of the organism into white rats. White rats are susceptible to plague infection and relatively resistant to *pseudotuberculosis*. The latter organism if examined in broth cultures after 16 hours incubation at 22 C is often motile whereas *Past pestis* is uniformly non motile.

Other organisms of the haemorrhagic septicemia group are *P. avispetica* of chicken cholera, *P. suis* of swine plague and *P. cuniculicida* of rabbit septicaemia and snuffles. Organisms of the Salmonella group (Gaertner paratyphus group) including the *Bacillus Danys* and *B. typhi murium* may also be sometimes encountered in rodents. In addition *B. mucosus capsulatus* (Friedlaender 1882) and *B. coli communis* have been encountered in them.

It therefore should be emphasized that rodents often may be infected with bipolar organisms that are not *Past pestis*. As an example of this the high mortality occurring among field rodents in and around De Aar South Africa in 1927 was at first recorded as due to plague infection. More careful examination however showed this to be erroneous. The same organism was isolated in Cambridge by Murray Webb and Swann and has been named *Listerella monocytogenes*. Nevertheless the disease was transmissible by inoculation or scarification of *Mamagua gerbilles* from the infected to healthy animals. Although this bacillus was especially fatal to gerbilles Scott (1939) points out that it was certainly not *Past pestis*. However Mitchell (1930) found a genuine *Pasteurella* infection among wild rodents in South Africa.

*Chronic Plague in Rats*—The Indian investigators have also called attention to the existence of chronic plague in rats. It is chiefly in the spleen and liver that the lesions consisting of abscesses occur thus differing from the acute plague in rats with milary nodules above described. In a series of 2,000 rats of the species *R. norvegicus* examined in Bombay 0.57 per cent showed signs of chronic plague. In the necrotic material plague bacilli could be found in approximately one half of these rats although frequently the bacilli were non virulent. It is possible that this chronic plague in rats may serve as the reservoir of infection which keeps up plague epizootics from year to year. Dordas (1922) in the examination of 5000 rats in the neighborhood of Paris found a number of animals with atypical lesions containing plague bacilli of lowered virulence. In some instances plague infection may be present in the rat without visible lesions. Plague in India according to White is less virulent now than formerly and this is attributed to a greater immunity of the rats.

*Epidemiology*—Plague is primarily a disease of rodents usually rats and man frequently contracts his infection from these animals. Over crowding in unsanitary lodgings especially predispose to epidemic outbreaks.

The disease may be classified clinically as bubonic septicaemic or pneumonic according to whether the lymphatic system the blood or the lungs are primarily involved bearing in mind however that in all cases of primary pneumonic plague the plague bacilli are present not only in the lungs but also in the blood and that in almost all cases of bubonic plague terminating fatally the plague bacilli appear in the blood shortly before death. In a small percentage (usually not more than 1%) of

The common human flea of Europe is *Pulex irritans* which is cosmopolitan in temperate regions of the United States *P. irritans* in California *Ctenocephalus canis* the dog flea and *C. felis* the cat flea in the eastern states. The species primarily responsible for the transmission of plague is *Xenopsylla cheopis* the Indian rat flea the commonest rat flea in the warmer regions throughout the world. Originally a parasite of the black (Indian) rat *Rattus rattus* it now equally infests the brown rat (*R. norvegicus*) in warm climates. It resembles *P. irritans* but is more yellow than brown in color. It also has a greater number of bristles on the head. *Ceratophyllus fasciatus* is the common rat flea of Europe and the United States. *Ctenocephalus canis* and *felis* *Leptopsylla musculi* and *Pulex irritans* have also been found frequently on both *Rattus rattus* and *R. norvegicus*.

**Fleas and Plague**—In 1897 Ogata infected mice by inoculating them with an emulsion of crushed fleas taken from plague rats. In 1898 Simond showed that if a rat dead of plague were placed in a large bottle and a healthy rat confined in a small cage introduced into the bottle and suspended above the dead rat so that there could be no contact between the dead and the living animal the well rat would contract the disease. If however the fleas were removed from the dead rat before the introduction of the caged rat no infection took place.

By reason of claims that the rat flea would not bite man these convincing experiments were in a measure disregarded. The complete confirmation of the correctness of this view as to transmission of bubonic plague was brought about by the Indian Plague Commission. In a large number of experiments it was shown that when healthy and plague infected guinea pigs were confined together in spaces where there were no fleas there were no plague infections of any of these well animals.

On the other hand in 35 experiments when fleas had access to the spaces plague infections were the rule. Again guinea pigs in cages which were suspended only two inches above a plague flea infected floor became infected but other animals which were suspended so high that the fleas could not jump up to them remained well. Two cages each containing a monkey were placed in a plague flea infected room. One was surrounded with a protecting zone of 6 inches of tanglefoot fly paper thus being the limit of the distance a flea can jump while the other cage was not so protected. The monkey in the cage without the sticky paper contracted plague while the second monkey remained well. It is usually when there is a great incidence of plague among rats that we have outbreaks of bubonic plague in man and it has been noted that the greater the epidemic the more heavily infected was the blood of the sick rats with the plague bacilli. It has been estimated that the blood of a rat dying with plague may sometimes contain as many as fifty million bacilli to the cubic centimeter although human blood rarely contains more than a million. A flea with a stomach capacity of about 0.5 cmm. could thus take in several thousand plague bacilli in a feeding on a rat whose blood was teeming with organisms.

Bacot has carried on some experiments which seem to show that fleas infected over a month previously and kept in a cool place could still transmit plague. This would indicate the danger from plague infected fleas which had been held in material packed away in boxes.

The plague bacilli ingested by the flea from the rat multiply in the alimentary tract of the flea. Infection in man may occur in several ways from the flea. The organism is passed in the feces of the flea and faecal pollution of the proboscis may occur at the time of biting or the faeces of the flea in the skin may be inoculated scratchingly when the flea bites or at the time that it is killed. Bacot and Martin also showed that infection of an animal might occur at the time of the sucking of the blood by the flea.

The body of the flea is flattened laterally. They may or may not have eyes and rows of conspicuous stout spines called combs which are of importance in classification. The puncturing apparatus of the flea consists of a pointed epipharynx and two distally serrated mandibles. These chitinous biting parts are contained in the labium which divides distally into two labial palps. The maxillae are conspicuous triangular structures and projecting farthest anteriorly are the conspicuous four jointed maxillary palps often mistaken for antennae. By the apposition of the internally grooved mandibles to the epipharynx a tube is formed through which the blood is sucked up. The antennae are inconspicuous and are in close apposition to the sides of the head behind the eyes and can only be well made out with a lens. Fleas have three pairs of legs and the male can be distinguished from the female by its smaller size and the conspicuous coiled up spring like penis within the abdomen. The female has a conspicuous gourd like spermatheca which varies in shape in different species. A very prominent structure is a pitted plate in the ninth abdominal segment (pygidium). Of importance in classification are prominent bristles originating from the seventh abdominal segment and projecting over the pygidium. These bristles vary in number and are known as antipygidial bristles.

The eggs are laid in the dust of floors, under rugs, or loosely on the hair or in the nests of their hosts. After 3 or 4 days a bristled worm like larva emerges from the egg. It has 14 segments and a distinct head with biting mandibles. The larvae do not suck blood but feed on any sort of organic material surrounding them. In this way probably they may ingest the eggs of some parasites, the larval stages of which develop later in the adult flea. If such an infected flea be taken into the mouth (of a dog, rat or child) parasitic infection may result. Some species require blood which they get from the semi-digested faeces of the adult fleas. After one to two weeks or more the larva forms a cocoon and develops into a nymph with three pairs of legs. The adult flea emerges after about three weeks. The whole cycle usually takes from one to three months. The adult fleas under favorable conditions may live from one to two years, but they die quickly in hot, dry climates. If cool and moist they may live for several months without feeding.

#### KEY TO FLEAS COMMONLY FOUND ON RATS AND CALIFORNIA GROUND SQUIRRELS

##### A With combs

###### 1 Eyes present

- (a) Combs along inferior border of head and on prothorax *Ctenocephalus canis* and *C. felis* (*Ctenocephalides canis*, *C. felis*)

###### (b) Combs only on prothorax

###### (1) Rostrum extending to trochanters

Prothoracic comb of about 18 spines

*Ceratophyllus fasciatus* (*Xosopsyllus fasciatus*)

###### (2) Rostrum extending well beyond the trochanters

Prothoracic comb of about 18 spines

*Ceratophyllus acutus* (*Diamanus montanus*)

###### (3) Rostrum scarcely reaching half the distance to the anterior coxae

Prothoracic comb of about 9 spines

*Hoplopsyllus anomalus*

###### 2 Eyes absent

- (a) Collar of combs on prothorax and four short ones along inferior border of head *Leptopsylla musculi* (*Ctenopsylla musculi*) (*C. segnis*)

##### B Without combs

- (a) Ocular bristle arises near upper anterior margin of eye. A line between this and the oral bristle approximately vertical. Two bristles posterior to antennae. A vertical ridge like thickening on mesonotum *Xenopsylla cheopis* (*Xenopsylla pallida*?) (Formerly *Pulex cheopis*)

- (b) Ocular bristle arises near lower anterior margin of eye. A line between this and the oral bristle approximately horizontal. One bristle posterior to antennae *Pulex irritans*

the sewer rat When the rat dies the fleas leave the dead body and seek a new host preferably one similar to the one just abandoned The sewer rat reaching the basement of houses and dying of plague is deserted by his fleas These will attach themselves to the house rats which range from basement to roof and later these dying are abandoned by the fleas which in the absence of a rodent host will feed on man and infect him

The black rat was probably introduced into Europe from India some time in the 1<sup>st</sup> Century and the great epidemics of the 14<sup>th</sup> and 15<sup>th</sup> Centuries followed As *Norvegicus* the sewer rat not being so much an associate of war replaced *Rattus* in Europe plague decreased or disappeared Also the flea which is especially harbored by the black rat is more prone to attack man

Today the house rat is rarely found in Europe while in many parts of the tropics it is common and in close association with man The fact that the sewer rat avoids the upper portions of houses may be another factor in the infrequency of plague epidemics in Europe at the present time where this rodent is common It was in former ages when the house rat was prevalent in Europe that the great epidemics

*Rattus norvegicus* is a stout build with a blunt nose and small opaque ears which barely reach the eyes when laid forward The tail is shorter than the length of the head and body together (39 per cent of such length) *R. rattus* is a delicately built rat with a slender head and sharp nose The ears are translucent and large and reach beyond the middle of the eye when extended The rather delicate tail is about 5 per cent longer than the length of the head and body taken together

In Madras there is practically an absence of *R. norvegicus* although *R. rattus* is present in numbers and the comparative freedom of the city from plague is striking

Mention has been made of the fact that wild rats present a variable degree of immunity to plague infection Sokhey and Chitre (1937) found in Nasik City with a plague rate of 36.34 per mille that none of 80 black rats from this locality died from experimental infection whereas in Madras City with only 0.3 deaths per mille 91.1 per cent of the rats died Scott (1939) reports that Bombay has not for 2 years shown a plague infected rat though 1000 are caught daily and brought to the laboratory for examination and its rats by experiment were highly immune with a 70 per cent susceptibility Nevertheless the human mortality has always been and is now high 99.8 per mille It is suggested that different races of *Rattus* now exist with different susceptibility In any locality the more susceptible die of infection leaving a higher proportion of naturally resistant animals

Mumford (1940) has called attention to the introduction of *Rattus attus alexandris* in the Pacific Islands which has been important especially in outbreaks of plague in Egypt Eskey (1934) has shown that the native *Rattus hawaiiensis* is an important agent in maintaining the rural epidemic type of plague in the Hawaiian Islands While 8 cases of human plague have been present there in the past two years 590 plague infected rats have been found in 1943

A guinea pig set free in a house suspected of harboring plague infected fleas is likely to become infected with plague if such fleas are actually present Such a measure has been frequently employed for the detection of plague infection in a house and the guinea pig has been termed a plague barometer The fleas would probably select the guinea pig as a host prior to man and the presence of such a rodent might to some degree thus be protective to man

Man is most commonly infected through the agency of the rat flea but fleas from other infected rodents may give rise to the infection or infection may occur occasionally from man to man through the agency of the human flea

Occasionally possibly also through the louse *Pediculus humanus* or the bedbug *Cimex lectularius* Philip (1939) has pointed out the possibility of ticks as vectors and Wayson and Eskey have found *Pasteurella* in ticks in the United States Several observers have recently emphasized the importance of the human flea in connection with the recent European human epidemics Bacot reported that of 34 varieties of fleas which had been found on rodents 21 species were probably transmitters of plague and with at least 11 of these species experiments demonstrating that they transmit



through regurgitation of the bacilli from its oesophagus and proventriculus into the wound made by the puncture \*

The species of rats which are most concerned in the spread of plague are *Rattus rattus* the black rat and *Rattus norvegicus* (*Mus decumanus*) the brown rat

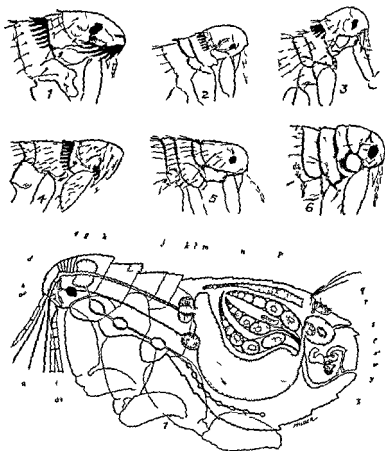


FIG 164—1 *Ctenocephalides felis* 2 *Ceratophyllus fasciatus* 3 *Hoplopyllus anomalus* 4 *Leptopyllus musculus* 5 *Xenopsylla cheopis* 6 *Pulex irritans* 7 Internal anatomy of flea (After Fox) (a) Maxillary palpus (a 1) pharynx (a 2) mandible (a 3) labial palp (a 4) maxillae (a 5) basal elements of rostrum and mandible (b) salivary pump (c) hypopharynx (d) aspiratory pharynx (e) muscles of the aspiratory pharynx (f) eye (g) oesophageal ganglia (brain) (h) thoracic ganglia (i) oesophagus (j) salivary duct (k) gizzard (l) salivary gland (m) stomach (n) aorta (o) ovaries (p) Malpighian tubules (q) pygidium (r) rectum showing rectal glands (s) anus (t) intestines (u) bursa copulatrix (u 1) ductus obturatoris (biliary duct) (v) retractor of penis (w) ducts of spermatheca (x) vagina (y) uterus (z) abdominal ganglia

**Method of Spread**—The spread of plague epizootics among rats seems to be rather by the fierce brown sewer rat, *Rattus norvegicus*. The more delicate black house rat *R. rattus* usually receives its infection from

Douglas and Wheeler (1943) confirmed this method of infection by experiments with *Dipodomys deserti* infections and rats

More recently Hirst points out that *Y. asi* must not be regarded as innocuous as it may cause outbreaks of plague in rats. Nevertheless it should be remarked that *Y. asi* has been found to be the predominant species in Ceylon and in the damp hot provinces of Madras and Lower Bengal where plague is never serious. It rarely bites man at temperatures above 80° F. C. Wu in a four year study in Shanghai found 85.5 per cent of the rats *R. rattus* but the prevailing flea was not *Y. cheopis*. More than 75 per cent of the fleas were *Lept. psylla musculi*. *C. conis* was next most frequent (17.6 per cent) and *Y. cheopis* was found only in the autumn and then in small proportion (6 per cent). Wu believes that plague has never gained a firm foothold in Shanghai and is not likely to do so unless factors develop favoring the prevalence and especially the seasonal prevalence of *Y. cheopis*.

The cat flea *C. nocephalus feli* is not only infectable with plague but is an active biter of man. Raybaud has found it as active in biting man as is *Y. cheopis*. On the other hand although *C. conis* is relatively cosmopolitan in its distribution it has not proven to be an important natural transmitter of plague. It has been demonstrated by Bacot and others that the flea after it has sucked the blood containing plague bacilli may sometimes remain infective for as long as from 29 to 47 days after biting.

**Meteorological Influences**—Plague today is found chiefly in the warmer latitudes. However extreme heat and dryness of atmosphere are inimical to its spread. In a temperate climate fleas are usually most numerous during the warmer seasons of the year hence bubonic plague is more common in the summer and autumn months. In the tropics it is most likely to become epidemic when the temperature ranges below 85° F. (30° C.). Temperatures of 68-77° F. are most favorable for multiplication and the activity of the flea. Temperatures over 85° F. are unfavorable to the development of the flea especially if the atmosphere is dry.

In India plague occurs during the cooler months of the year also when the mean temperature is below 85° F. (30° C.) and the air has a high relative humidity or as it is sometimes expressed a low saturation deficiency.

White believes that humidity is the most important factor in the spread of plague in India. The general curve of the disease for British India shows a normal seasonal rise in January with the peak at the beginning of March and the minimum in June. Usually a secondary rise takes place in August. Rat fleas have been found most prevalent in India during the months of February to May inclusive. The infestation of *R. norvegicus* has frequently been more than double that of *R. rattus* the number of fleas per rat during these months being about 5 for *R. rattus* and 12 for *R. norvegicus*. The length of time the fleas remained infected with the plague bacillus depended upon several factors chief of which was temperature and humidity. During one epidemic in Bombay the rat fleas remained infected for about 15 days but during the non epidemic season this time was reduced to 7 days.

In Egypt Peirce and Todd especially studied the influence of the seasons on the spread of the disease. In the southern provinces the maximum incidence was in February and March but in northern Egypt at Alexandria and Port Said it was in July. The optimum temperature of the epidemic spread was between 20 and 25° C. This temperature and the high relative humidity were most favorable for the development of the fleas. On the other hand Otten (1932) found that in the mountainous districts of Java the fleas reached their maximum in October and November and that

plague infection have been performed. In man the species usually causing infection have been *Yenopsylla cheopis*, *Ceratophyllus fasciatus* and *Pulex irritans*.

The principal rat flea of the Orient is *Yenopsylla cheopis*. It is the special flea of *Rattus rattus* abounding in tropical countries and it is this species of flea that is prone to attack man and to transmit infection from rodent to man. This flea is without combs like *Pulex irritans* the human flea but is of a lighter color and has an ocular bristle near the upper margin of the eye and two bristles posterior to the antennae. In Europe and the United States *Ceratophyllus* (*Dasopsyllus*) *fasciatus* is the common rat flea of *R. norvegicus*. It is a capable transmitter of infection among rats but is not very prone to attack man. Rondebush and Becker (1934) have shown that the tropical rat flea *Y. cheopis* has established itself in the East, Middle West and Far West in the United States. Ewing and Fox (1938) think there is a possibility that a more resistant race of this flea has been developed in some of our northern ports such as New York and Boston. However Rondebush (1939) emphasizes that such a view is not necessary to account for the survival of the flea in the colder temperatures of temperate zones. It matters not how low the temperature falls as long as the flea, its larvae and eggs remain in the warm confines of the rat's tunnel. The same warmth which allows the rat to live can also keep the flea alive. Eskey (1938) has found that of all fleas tested in the United States *Y. cheopis* contracts plague infection much the most easily, the proportion being 55 per cent for *cheopis* and only 21 per cent for all other fleas.

*Pulex irritans* has recently been found in nature on susceptible wild rodents and Jellison and Kohls (1936) suggest that it may play a part in future epidemics in the nearby human population in California, Oregon and Montana. Its role in the Parisian epidemic has been referred to. Also on epidemiological grounds Eskey believes that *P. irritans* is probably responsible for most of the human cases of plague in the high mountain districts of Ecuador where *Y. cheopis* does not occur. Blanc and Baltazard have demonstrated the importance of *P. irritans* as a vector in the epidemic in Morocco in 1941. The fleas were taken from the plague patients and fed upon rats which succumbed to the infection.

Many other species of fleas may also transmit plague. In East Africa, Kenya and Uganda, another species of *Yenopsylla*, *Y. brasiliensis* was found by Kauntze (1935) to be a more important vector than *Y. cheopis*. Moreover it is the flea found commonly in the huts. In 1934 in an outbreak of plague in the Argentine Pampas no rats and no *Y. cheopis* were found but there was an epizootic among a wild rodent *Geomys griseoflavus* almost ubiquitous there and of arboreal habits. The prevailing flea was *Rhopalosyllus occidentalis* and the rodent flea index was as high as 6. While *Ceratophyllus fasciatus* is a relatively good transmitter of experimental plague nevertheless in some localities, as in Australia and Europe where it prevails, Hirst points out that its period of maximum seasonal prevalence falls outside the plague season.

In California and Oregon the ground squirrel *Citellus beecheyi* has become infected and may transmit the disease by its fleas *Ceratophyllus acutus* and *Hypopsyllus anomalus*. Eskey and Haas have shown that 13 species of fleas from North American rodents may be infected and transmit plague by their bites. In Argentina and Ecuador *Rhopalosyllus cunicola* of the cavity in South Africa *Yenopsylla eridos*, *Dinopsyllus lypsus* and *Chiasopsyllus rossei* of the gerbille and the multimammate mouse as well as other species of fleas on wild rodents have been incriminated as the natural transmitters.

In the residual area in Transbaikalia *Ceratophyllus isolatus* on the tarbagan and *C. tesquorum* on the ground squirrel are involved.

Hirst and Cragg have independently suggested that another species *Yenopsylla asiatica* which commonly infects rats in certain parts of India does not bite man with avidity and is probably not concerned in the transmission of human plague. Hirst also states that in his experiments he has been unable to transmit plague from rat to rat by means of *asiatica*. It would however be very dangerous to disregard the capability of *asiatica* to convey plague infection particularly since the very closely allied species *Yenopsylla cheopis* is one of the most common transmitters of plague.

dead or dying rodents. On the Gold Coast the giant rat *Cricetomys gambianus* and in Kenya the field rat *Vicentinus abyssinicus* are important.

In the Argentine one of the Cricetidae *Graomys griseofuscus* has been found especially infected. Moll (1941) believes the guinea pigs kept in the houses of Indians in Ecuador and eaten by them as food are responsible for the maintenance and spread of plague infection.

At the Calcutta Conference in 1934 it was pointed out that epizootics in the Cumbrum Valley were often preceded by death among bandicoots *Peromyscus* followed by high mortality among rats and mice.

**Other Means of Infection**—Human infection however is not always transmitted by fleas. In a small percentage of the bubonic cases infection occurs from exposure of abraded surfaces of the skin to the plague bacillus. Instances of such infection have occurred in barefooted individuals with small wounds of the feet from walking on floors or stepping on material infected with plague bacilli or through abrasions on the hands of those who have performed autopsies on or handled the bodies of those who have died of plague or who have shot and skinned rodents infected with plague.

Infection in primary human septicaemic plague is usually acquired through the mucous membranes particularly of the mouth and throat and the conjunctivae. Particles of infected sputum which have been accidentally coughed into the eye have produced human septicaemic plague. Animals such as monkeys may be given primary septicaemic plague by instilling a few drops of a culture of *Bacillus pestis* in the eye or by rubbing a small amount of the culture on the mucous membranes of the gums without producing visible erosions. Infection of the mucous membranes of the mouth may occur also in man through the hands conveying infection as might occur in individuals who have shot or skinned infected rodents.

There has been an outbreak of septicaemic plague reported in Ceylon in which there was an absence of plague in rats. The infection was possibly transferred directly through human fleas or bedbugs.

In epidemics of primary pneumonic plague infection does not occur as in bubonic plague through the agency of heavily infected fleas or through the skin but directly from man to man aërially through droplets of infected sputum expelled by coughing as was conclusively shown by Teague and the writer in the Manchurian epidemic. In no other infectious disease have such enormous numbers of uniformly highly virulent microorganisms been demonstrated in the droplets of sputum coughed up by patients with primary epidemic plague pneumonia.

The influence of the environment and temperature is also of importance in the spread of pneumonic plague. Taggart in connection with our Manchurian studies (1913) especially emphasized that atmospheric temperature is an important factor in determining the spread or failure to spread of pneumonic plague. Later working in Manila with Barber it was pointed out that fine droplets containing plague bacilli remained longer in a chamber atmosphere with a very small water deficit such an atmosphere under ordinary circumstances being of common occurrence in very cold climates as Manchuria in winter whereas it is extremely rare in warm ones.

The writer found in Manchuria that the plague bacillus will remain not only viable but fully virulent after weeks in frozen sputum or in frozen corpses. When such sputum becomes frozen and pulverized it may be blown about and remain infective for

human plague reached its maximum in December. The flea index followed the rise and fall of the saturation deficiency which is contrary to what usually occurs in India. Rogers believes that the decline of plague in India during the height of the hot season is due to high temperature and high saturation deficiency. In general sunlight and drying are the especially inimical factors for the development of *P. pestis* and the dry seasons are unfavorable for the spread of plague.

Pneumonic plague not being spread by the flea is obviously not influenced by temperature in this way, though cold temperatures favor the outbreaks of large epidemics. The bacillus of plague can withstand freezing temperatures for months.

*Sylvatic plague* is the term which has been applied to plague occurring as an epizootic among wild rodents in wooded or rural districts, usually not inhabited by man and where there has been no knowledge of the human disease. In sylvatic plague subacute and chronic infections have been encountered as well as latent plague infection. Thus the tarbagan when contracting the plague in the autumn may harbor the virus during hibernation and in the ensuing spring the infectivity may be renewed and the animal may start an epizootic. In some instances lymph nodes have been found swollen in rodents without other obvious symptoms or gross pathological changes. (See below page 675.)

Recently in California a method of discovering occult plague was employed in which fleas were collected from ground squirrels in the field killed by chloroform and suspended in physiological saline and later inoculated into other laboratory animals. Though it is said the squirrels themselves showed no evidences of plague inoculations of the fleas into other rodents were said to give rise in some instances to septicæmic plague. Esbey (1939) found that fleas killed with hydrocyanic gas were much more infective for guinea pigs than if killed with chloroform or ether.

Sylvatic plague is dangerous to man only when he enters these regions populated by wild rodents and exposes himself to their fleas or handles and kills the infected animals.

Wu Lien Teh (1936) gives a list of 72 rodents other than domestic rats and mice which are said to suffer from plague in nature. However a number of these have not been demonstrated to be of great importance.

In California especially *Citellus beecheyi* the ground squirrel and fourteen other species are infected. In this region also the tree squirrel chipmunk marmot and prairie dog have all been found infected.

In Manchuria *Arctomys bobac* the tarbagan and several other smaller species of rodents and in South West Russia *Spermophilus musicus* and other spermophiles have played important roles in causing infection.

In West Africa Leger and Baurý found that the shrew *Crociotura stamphii* played a role in the Dakar plague epidemic. In South Africa on the high veld the gerbilles *Taterona lobengulæ* and *Desmodillus auricularis* the ground squirrel *Geosciurus capensis* and the small mouse *Mus mus coucha* have recently been infected and these rodents have shown a very high mortality over a wide area. The domestic multimammate rodent *Mastomys coucha* seems to be the host by which the infection on the high veld is transmitted to man which itself may become infected by the wild gerbille *Taterona lobengulæ*. In the lower bush country in South Africa the striped mouse *Rhodomys pumilio* plays the chief role while the springhaas *Pedetes capensis* a giant jerboa on account of its extreme mobility is capable of widely disseminating plague. Two carnivores the sunskate and the yellow mongoose are susceptible to plague by feeding on

primarily involved. Monkeys are very susceptible to plague but no epizootics among them have been recorded.

*Age sex race and occupation* are not predisposing factors of importance in connection with plague. Both the young and old are equally susceptible. During epidemics more cases occur between the ages of 20 and 40 but this is due particularly to increased exposure to infection during these years of life.

In some outbreaks more cases have occurred in women but there again the habits of the women in living more continuously indoors under unhygienic conditions seems to have been a factor of greater importance than sex in connection with the incidence of the disease. In the Manchurian epidemic of pneumonic plague the women and children were not so frequently exposed to infection and the number of cases of pneumonic plague which occurred in women and children as compared with the number in men was comparatively small. It is true that more cases of bubonic plague occur among

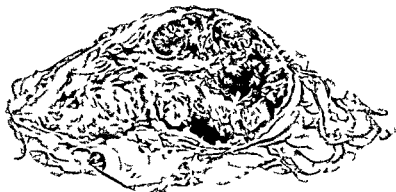


FIG 165—Cutaneous bubo (Govt Biological Museum)

natives in the tropics than in Europeans in such localities or than in individuals in temperate climates. This is dependent particularly upon the fact that the natives in the tropics are generally lightly clad and more exposed to the bites of fleas and that likewise they generally dwell under more unhygienic and unsanitary conditions. The apparent immunity of some races in endemic districts is largely due to the good hygienic conditions under which they live.

### PATHOLOGY

It is rare that one finds the primary vesicle marking the site of entrance of the plague infection. Thus in 13 cases where plague was contracted by direct cutaneous inoculation of those performing autopsies on plague victims only two showed evidences of local reaction as shown by the formation of a primary vesicle.

The chief points noted in a plague autopsy are (1) The marked involvement of the lymphatic system as shown by intense congestion and haemorrhagic oedema of the lymphatic glands. Not only are the glands involved tributary to the site of inoculation thus forming the primary bubo but there is secondarily more or less inflammatory change in many of

considerably longer periods of time than when in the form of moist droplets. Isolated or small groups of cases of pneumonic plague (more commonly of pneumonic plague secondary to bubonic) have since the Manchurian epidemic occurred in various parts of the world generally in tropical but sometimes in temperate climates. However under the environmental conditions where these outbreaks occurred the disease has not assumed epidemic proportions.

One of the most important of these small outbreaks occurred in California in the winter of 1924. The first case in the outbreak was of bubonic character. Subsequently 32 cases of pneumonic plague developed rapidly from contact, all terminating fatally. The immediate sanitary measures undertaken by the efficient health authorities prevented the further spread of the disease.

The conditions in winter in Manchuria which cannot be described in detail here still greatly favor outbreaks of primary pneumonic plague.

It has been suggested recently that in such epidemics the plague bacillus must act in symbiosis with another organism in order to bring about such epidemic manifestations but there is no evidence that this is true. Norman White states that he has come to the conclusion that the plague bacillus alone does not and cannot cause widespread epidemics of pneumonic plague. Anyone however who is willing to take the risk can easily demonstrate that by spraying pure cultures of highly virulent plague bacilli free from any other organism or bacteriophage there can be produced in guinea pigs, monkeys or tarbagans who breathe the atmosphere containing the plague bacillus, outbreaks of typical primary plague pneumonia as observed in human beings. As has been pointed out by Petrie during these severe epidemics, an essential requisite for the spread of the infection is the close contact between the sick and the healthy that results from overcrowding or from the habits of those exposed to risk. Outbreaks of pneumonic plague not uncommonly take their immediate origin from patients with bubonic or septicæmic plague in whom a secondary pneumonia has supervened, such a case causing a primary pneumonic infection in another individual.

The possibility of carriers of plague bacilli in those who might go on to convalescence need not be considered as practically all cases of primary epidemic plague pneumonia die. Other material from the patient than sputum does not seem to be a source of danger in the spread of plague so that there is no need for the general disinfection of faeces. In a few instances however the urine may become secondarily infected. Ogata has called attention to this and the Plague Research Commission found in a septicæmic case of plague that the urine was infected and killed a guinea pig when inoculated subcutaneously.

With reference to the development of primary pneumonic plague a history of association with another patient with symptoms suspicious of plague infection or a history of having shot or handled or skinned a possibly infected rodent may often be obtained, the infection being transmitted by the hands to the mouth and thence to the larynx and bronchi. Absence of proper ventilation, overcrowding and close contact between the sick and healthy as well as low atmospheric temperature are predisposing factors of great importance in the spread of primary pneumonic plague.

In bubonic plague also the social and hygienic conditions of the patient are often important predisposing factors and the disease is particularly associated with filth, absence of proper ventilation, overcrowding and the parasitic accompaniment of such conditions.

It is a matter of experience that the transference of plague from place to place generally occurs from infected rats or infected fleas which have been transported by ships though sometimes by rail and other conveyances. A case of bubonic plague in a ward with other patients would not be a source of danger provided there was freedom from fleas and that no plague patient developed a secondary pneumonia. It is very doubtful as to human infection ever taking place by way of the alimentary canal although there is some evidence that rarely the tonsil may be

plague in Ceylon in cases where plague bacilli were demonstrated in smears and cultures from spleen and blood. Castellani noted especially meningeal congestion and some splenic enlargement.

### SYMPTOMATOLOGY

**Incubation Period**—The incubation period of human plague varies usually from 2 to 10 days but is generally from 3 to 4 days. In primary pneumonic plague it may not be over 2 or 3 days.

**Symptoms and Course of Bubonic Plague**—In bubonic plague premonitory symptoms are not usually observed though occasionally there may be 1 or 2 days of malaise and headache. The onset except in mild cases is usually abrupt with fever commonly accompanied by a moderate rigor or repeated shiverings. The temperature rises rapidly to 103°F or 104°F sometimes even reaching 107°F. The pulse becomes rapid and

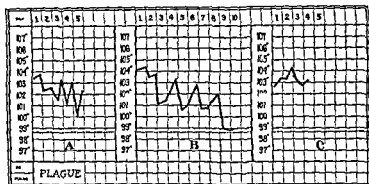


FIG 167—A Temperature chart of fatal case of bubonic plague. B Chart of case of bubonic plague going on to recovery but with suppuration of plague bubo. C Chart of fatal case of pneumonic plague.

the respirations increased. There is headache which is usually severe and mental dullness and this condition is generally followed by mental anxiety or excitement. The patient may become maniacal. The skin is hot and dry, the face bloated, the eyes injected and the hearing dulled. The tongue is usually swollen and coated with a creamy fur or later with a brown or black layer. The symptoms usually complained of within the first 24 hours are very severe headache and backache, burning in the throat or stomach and nausea and vomiting may occur. Constipation is present as a rule. The pulse is either very small and thread like or full and bounding. At times there may be acute delirium at others lethargy and coma. In children convulsions usually occur. The urine is scanty and generally does not contain more than a trace of albumin and no casts. Later in the disease the albumin may increase somewhat. The high febrile stage lasts from 2 to 5 days or longer. The decline in temperature may be sudden or gradual. Cases that do well usually show a gradual fall of temperature and after 14 days the temperature may be



the lymphatic glands of the body. There is also a marked periglandular oedema with haemorrhagic extravasations of the connective tissue surrounding the primary bubo, this mass being made up of a group of glands matted together by this periglandular exudate.

(2) The marked destructive effect of the toxine of the plague bacillus upon the endothelial cell lining of blood vessels as well as of lymphatic ones. This causes the extensive blood extravasations so characteristic of plague as shown by petechial spots, not only of the skin but of the serous and mucous membranes as well throughout the body.



FIG 166 — Hyaline fibrin thrombi  
(Govt Biolog Labs Manila)

There is a general congestion of all organs of the body. The meninges of the brain are deeply congested and there may be haemorrhagic extravasations in the brain substance itself. However meningitis has been reported only in a few cases. The spleen is generally markedly congested and enlarged to 2 or 3 times its normal size. There may be haemorrhagic extravasations throughout the spleen pulp. The bacilli are chiefly scattered throughout the venous sinuses. There is also active congestion of the liver. The kidneys are intensely congested, haemorrhages beneath the capsule are usual, and we often find hyaline fibrin thrombi in the tufts of the Malpighian bodies as was emphasized particularly by Herzog in Manila. The plague toxin has a marked effect on the cardiac muscle so that we usually find dilatation of the right side of the heart with fatty degeneration of the muscle fibers. In a study of the pathology of primary

pneumonic plague, Strong noted pericardial and pleural ecchymoses with fibrinous pleurisy over the affected lung areas. The process was at first lobular, but later might involve the entire lobe. There was marked congestion of the bronchial mucosa with involvement of the bronchial glands. The larynx and trachea are also intensely congested. Microscopically there is a distension of the alveoli and bronchial passages with a haemorrhagic exudate. There is practically no fibrin in the alveolar exudate. The process seems to extend by continuity along the bronchi and bronchioles. Plague bacilli pack the exudate found in the bronchi and bronchioles. In a report on the autopsy findings of septicæmic

seen in rodents. Leger and Baury found three apparently healthy individuals in a plague district who had moderately enlarged inguinal glands which were not inflamed or tender. However in the fluid from these glands plague bacilli were demonstrated by microscopical examination and animal inoculation. They point out that such patients may serve as carriers. In Brazil Macchuavello (1941) has described a disease known as cold bubo (*ingua de frio*) or stone fever in which the bubo has a tendency to become ligneous and to be absceded or to recur. The condition has been proved bacteriologically to be plague. Kamal (1941) found in an epidemic in Egypt a number of children with large glands sometimes tender sometimes painless but with no other symptoms. He regarded these as cases of inapparent or ambulatory plague.

**Symptoms and Course of Pneumonic Plague** — The onset of the disease is usually somewhat abrupt prodromal symptoms are rare. The disease usually begins with chilly sensations but a distinct rigor is unusual. Epistaxis is also rare. There is headache loss of appetite an increase in the pulse rate and fever. Within from twenty four to thirty six hours after the onset the temperature usually has reached 103 F or

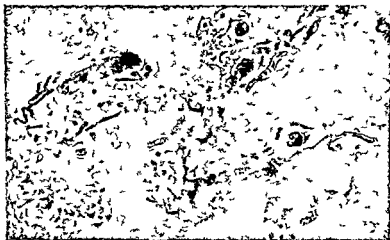


FIG. 69.—Bacteriologically confirmed plague showing the presence and form of *Y. pestis*.

104 F and the pulse 110 to 130 or more beats per minute. Cough and dyspnoea appear within twenty four hours after the onset of the first symptoms. The cough is usually not painful. The expectoration is at first scanty but soon becomes more abundant. The sputum at first consists of mucus which shortly becomes blood tinged. Later the sputum becomes much thinner and of a bright red color it then contains enormous numbers of plague bacilli in almost pure culture. The typical rusty sputum of croupous pneumonia was not observed. The conjunctivae become injected and the tongue coated with either a white or brownish layer. The expression is usually anxious and the face frequently assumes a dusky hue. Labial herpes is very uncommon. The patients sometimes complain of pain in the chest but usually this is not severe. Apart from the disturbances due to the dyspnoea and their anxiety for their condition they usually appear to suffer but little and usually do not complain of pain. In the later stages of the disease the respirations become greatly increased and the dyspnoea usually very

subnormal Buboes inflammatory enlargements of the lymph glands are sometimes the first sign to attract attention by their pain They more often make their appearance from the second to the fifth day after the onset of the fever The temperature frequently shows a decline when they appear The affected gland is often hard and painful to the touch In fatal cases, it may retain these characteristics, in others it suppurates The average size of the bubo is from a walnut to an egg Buboes appear in 75 per cent of the cases In the cases in which buboes are present, they occur in the inguinal glands in approximately 65 to 70 per cent in the axillary 15 to 20 per cent and the cervical, 5 to 10 per cent Carbuncles appear in about 2 per cent in which there are reddened indurated patches of skin which subsequently necrose The spleen is



FIG 168 —Plague buboes (After Deutman)

frequently moderately enlarged, but often cannot be palpated Haemorrhages from the stomach and intestine are not uncommon and when the disease is complicated with the pneumonic form they may occur from the lung Epistaxis is also not infrequent The blood usually shows a leucocytosis of forty thousand or more the increase being in the polymorphonuclear leucocytes The plague organism can be isolated from the blood in about forty five per cent of the bubonic cases

**Course**—The attack of high fever lasts generally three to five days or longer but the patient may die earlier If however he lives for five days there is greater chance of recovery If the bubo suppurates recovery may be delayed from two or three weeks to a month

**Mild Cases of Bubonic Plague Pestis Minor**—In this type of plague there may be often very slight fever or prostration with swelling and tenderness of the glands of one groin more rarely of one side of the neck or axilla The glands may or may not suppurate and the developing bubo may resolve Sometimes the patient is not sick enough to go to bed and these cases are not infrequently ambulatory in character In the more chronic forms the buboes are often indolent and may last two or more months In microscopical preparations made from them small numbers of plague bacilli the majority of which have degenerated and undergone involution are frequently found These cases correspond in some respects to the subacute and chronic forms of plague

**Symptoms and Course of Septicaemic Plague**—Septicaemic plague may occur during the course of bubonic plague always occurs in pneumonic plague and may occur as a form of primary infection. When primary septicaemic plague results the infection has usually occurred through the mucous membrane of the mouth and throat death resulting from septicaemia before macroscopic lesions are visible in the lymphatic glands or lungs. Nevertheless at autopsy at least some of the lymphatics are usually found to be enlarged congested and even haemorrhagic and in a few instances early buboes may develop shortly before death.

In this form the nervous and cerebral symptoms often develop with great rapidity and intensity and the course of the disease is very rapid the bacilli appearing in the blood almost at the onset of severe symptoms. The attack usually begins with trembling and rigors intense headache vomiting and high fever. The countenance usually depicts intense anxiety. Extreme nervous prostration restlessness rapid shallow respirations and delirium are common symptoms. In some cases the cardiac symptoms are the most prominent. The patients soon pass into a comatose condition and die sometimes within 24 hours of the onset of the attack but sometimes not until the third day. Cases of primary septicaemic plague are always fatal. Haemorrhages from the intestine sometimes occur in this form of plague as well as in bubonic plague. There is no distinct evidence that such cases are of primary intestinal origin. Haemorrhages from the nose and kidneys are also not uncommon.

#### DISCUSSION OF SYMPTOMS AND PATHOLOGICAL CONDITIONS IN DETAIL

**Circulatory System**—The plague bacillus produces a powerful endotoxin which often causes a dilatation of the arteries lowering of the blood pressure and alterations in the functional activity of the heart as well as degenerative changes in the heart muscle. It also acts particularly upon the endothelial cells of the blood vessels and lymphatics the inflammatory reaction frequently causing circulatory obstruction. One of the most characteristic features of the pathology of plague is the tendency to produce general dilatation and engorgement of the vessels with cutaneous subserous submucous parenchymatous and interstitial haemorrhages. In patients who have died of plague the most common of the latter are in the epicardium the pleura peritoneal surfaces the stomach and intestines and the mucosa of the stomach and small intestine. Sometimes extensive haemorrhages are found in the peritoneal mediastinal or pleural cavities. In the kidneys there are frequently subcapsular and renal haemorrhages and blood extravasation into the pelvis of the kidneys and ureters as well as in the bladder and generative organs.

Sometimes there are considerable extravasations of blood into the substance of the brain. In bubonic plague numerous haemorrhages are almost always present in the bubo. The tissues are characterized by vascular dilatation and engorgement followed by oedematous infiltration the effect of the toxin being evident on the vessel walls. The endothelial cells become swollen proliferated and degenerated. Later hyaline degeneration of the walls may occur.

During the clinical course of the disease haemorrhages are frequent. The bleeding may take place from the nose mouth lungs stomach or

marked, the patients frequently gasping for air for several hours before death. Cyanosis is then common.

The signs of cardiac involvement are always marked in the advanced cases, the pulse becoming gradually more rapid, feeble, and running, finally it can not be felt.

Gallop rhythm of the heart sounds is frequently observed. Death frequently occurs from cardiac paralysis and exhaustion.

The patients succumb after slight physical exertion such as sitting up in bed to take nourishment or on being moved. A few hours before death the temperature often declines to below normal. Delirium and coma are frequently present before death.

The urine in the later stages may show the presence of albumin. The diazo and indican reactions have not been observed in the few cases in which the urine was tested. Extravasations of blood have been found in the pelvis of the kidneys at postmortem examination.

The spleen is usually not palpable and the lymphatic glands not enlarged. Petechiae are occasionally seen but larger haemorrhages of the skin are usually not present. Bloody diarrhoea is occasionally observed. Plague bacilli frequently may be present in the blood in such numbers that a simple microscopical examination suffices for their detection; in other cases cultures are necessary for their discovery. A marked leucocytosis may occur though in some very severe cases the leucocytes are not increased.

The physical signs in the lungs are often slight even in cases well advanced in the disease. On percussion dullness is often absent and the vocal fremitus and resonance unchanged. In a small proportion of cases however smaller or larger areas of dullness may be discovered. On auscultation râles are frequently not present except shortly before death. When present early in the disease they are usually of the fine variety. Numerous moist râles are heard late in the disease and are due to the oedematous condition of the lungs. The character of the râles is in accordance with what one would expect from the condition of the lungs and bronchi and the character of the exudate observed

FIG. 170.—Cellulo cutaneous plague. (After photo of Institute of Tropical Hygiene, Amsterdam.)

at necropsy. Coarse râles such as occur in cases of catarrhal bronchitis usually are not present. Feeble respiratory sounds, tubular modification or pure tubular respiration over small areas are the conditions found most commonly on auscultation. Not infrequently a dry pleuritic rub is heard.

The limits of dullness of the heart are sometimes increased to the right of the sternum. The heart sounds are rapid and usually become feeble or embryocardiac in character toward the end. In the early stages the secondary pulmonic sound may be accentuated but it soon becomes much less distinct.



**Blood** —Early in the disease there is no reduction in the number of red blood corpuscles or in the percentage of the haemoglobin. In fact both Rogers and Castellani have observed that the red cells and haemoglobin are not infrequently increased above normal. In the late stages of bubonic plague particularly in the cases with complications a moderate secondary anaemia may occur. A leucocytosis is almost invariable in bubonic plague except in the mildest cases during the first three days of the disease. Usually the count is in the neighborhood of from twenty to twenty five thousand. In about five per cent of the cases it may be higher the leucocytes occasionally numbering forty thousand or even more. A differential count will show that the polymorphonuclear leucocytes are found to be increased and the large mononuclear cells usually diminished. In some of the very rapidly fatal septicaemic and primary pneumonic cases in which collapse and death appear early there may be no leucocytosis. In such cases plague bacilli may sometimes be present in the blood in such numbers that a simple microscopical examination of a hardened and stained specimen suffices for their detection. The plague bacillus can be cultivated from the blood in the primary septicaemic and primary pneumonic cases as well as in about one half of the bubonic cases of plague. The plague bacilli after they appear in the blood in bubonic plague increase up to the time of death and they can always be cultivated from the blood at autopsy. Over ninety per cent of the cases of bubonic plague in which the bacilli appear in the blood terminate fatally.

**Buboes** —Buboes or inflammatory swellings of the lymphatic glands which develop in about three fourths of the cases of plague may become noticeable any time from the onset of the attack to the fifth day. More often they develop within forty eight hours of the onset of the fever. Usually they increase rapidly in size.

At first a single gland may be felt enlarged but more commonly several adjacent glands are involved. Sometimes groups of glands become successively infected in which case there is always more or less periglandular infiltration. Thus a bubo in the inguinal region not infrequently extends into the iliac region affecting the lymphatic glands of the abdominal cavity and forming secondary buboes which can sometimes be felt as a mass through the abdominal wall. This condition has been mistaken for an appendicular abscess. The buboes vary greatly in size more commonly they are about the size of a walnut but they may be as large as an egg or even an orange. They are usually single but in about ten to twelve per cent of the cases they may be multiple and form on both sides of the body. As has been emphasized the buboes form in the inguinal region in from about sixty to seventy per cent one or more of the inguinal or femoral glands being involved. In about fifteen to twenty per cent they occur in the axillary region where the bubo often occludes the axillary space and obliterates the outline of the margin of the pectoralis major. In this region there is usually extensive inflammatory exudation which extends over the side of the chest and sometimes upwards to the shoulders and even to the side of the neck. Such cases frequently result fatally and cases with axillary buboes often become septicaemic early in the disease. In about five to ten per cent of the cases the bubo forms under the jaw or at its angle more rarely elsewhere in the neck or in the tonsils. In these situations there is often much oedema and exudation especially in the vicinity of the bubo and the patient may die from suffocation the trachea and glottis first becoming erythematous. In some instances in which the buboes have occurred in the tonsils cases have been mistaken for diphtheria.

kidney and sometimes from the uterus and bladder. These haemorrhages generally occur in severe cases of the disease. On examining the skin small punctiform haemorrhages from about 1 to 2 millimeters in diameter are sometimes observed scattered over the skin in greater or less profusion. The petechiae may occur on the face, neck, chest, abdomen or extremities. Sometimes larger patches of ecchymosis in the neighborhood of 1 centimeter in diameter are observed in the skin. Larger cutaneous effusions of blood are rarely seen, except at autopsy. The purpuric haemorrhages in bubonic plague usually do not appear before the third day of the disease. However, in septicaemic plague they may be seen earlier.

**Heart.**—At autopsy the right side of the heart and the great veins are usually distended with fluid or only partially coagulated blood. During the disease the patient frequently experiences a feeling of oppression over the precordial region. The heart sounds at first are clear and the second pulmonic sound may be accentuated, but as the disease progresses they become feebler or embryocardiac in character and the first sound may be no longer heard. Sometimes heart failure may occur without any other sign of collapse. It may occur following exertion such as sitting up, but it sometimes takes place while the patient is lying in bed. In primary septicaemic plague the course of which is very rapid the cardiac symptoms are frequently the most prominent ones. In pneumonic plague the limits of dullness of the heart are sometimes increased to the right of the sternum. At onset the second pulmonic may be accentuated but it soon becomes indistinct. As the disease progresses gallop rhythm may occur. Death takes place usually from cardiac paralysis and exhaustion.

**Pulse.**—The pulse in bubonic plague varies greatly. More commonly at the onset of the disease it is full and bounding, 100 to 120 per minute, becoming later still more rapid, 120 to 140 per minute, small, thready, irregular and often dicrotic. However in some cases it is small and thread like and very rapid from the onset of symptoms. In cases likely to prove fatal the pulse becomes so rapid and thready that it is impossible to count it. In such cases however the larger arteries can often be observed to pulsate forcibly. In mild cases of plague the pulse may only show slight acceleration.

**Temperature.**—The temperature curve in plague is often very irregular and not characteristic. In the severe cases the initial rise is usually rapid and may be anywhere from 103 F to 106 F. The temperature may reach its highest point on the evening of the first day of fever but usually the height of the curve is not reached till the close of the second or third day. From the third to the fifth day there is usually a remission of several degrees. Later the temperature may again rise and in fatal cases it may reach 107 F before death. A sudden fall of temperature during the height of the disease with a collapsed condition sometimes occurs and usually also indicates a fatal issue. In more favorable cases after the secondary rise the temperature often falls slowly and gradually with more marked remissions each morning until the normal or even subnormal point is reached. The course of the fever often lasts in uncomplicated cases from 6 to 12 days. Suppuration of the buboes however may cause great irregularity of temperature and the occurrence of complications may considerably prolong the period of fever. As a rule the higher and more continuous the temperature the ever the other symptoms. In mild cases of bubonic plague the temperature may fall to normal as early as the second or third day and it may not reach over 100 F during the attack. In primary septicaemic plague the temperature usually rises suddenly and remains high until death supervenes. Occasionally however if the patient lives from forty-eight to seventy-two hours after the onset the temperature may fall suddenly reaching normal or becoming subnormal just before the fatal outcome. In primary pneumonic plague the onset of the temperature is rapid and reaches the maximum point usually within twenty-four to thirty-six hours. In this form of the disease the temperature also often declines to below normal before death.

be formed in the lungs. In addition pneumonia in bubonic plague may occur as a result of infection with *Diplococcus pneumoniae* and in some of these lesions both the diplococcus and the plague bacillus may be encountered. In the metastatic form of pneumonia it is frequently very difficult to recognize the condition clinically. Occasional crepitant râles may be heard over small areas. In such cases the rapid decline in the general condition of the patient may suggest the condition. However if the lesions are sufficiently extensive in the lungs plague bacilli may sometimes be found in the sputum.

**Urinary System.**—The kidneys are usually markedly affected in plague. Congestion and parenchymatous degeneration are almost always present. Extensive haemorrhages may occur in the pelvis of the kidneys ureters or bladder. Microscopically profound cloudy swelling of the epithelium of the uriniferous tubules with the presence of granular or hyaline material in the latter is almost always present in fatal cases. A very characteristic change in the kidneys in plague sometimes observed is the presence of hyaline fibrin thrombosis of the glomerular capillaries. A lesion which was first emphasized in Manila by Hezog (1909). These lesions explain in a general way the urinary disturbances which may be observed clinically. The urine is usually diminished in quantity of a high color sometimes smoky and of high specific gravity. It usually contains a moderate amount of albumin but albumin is not always present in the less severe cases. The urea uric acid and chlorides are often decreased. Microscopically epithelial cells pus cells and sometimes red blood corpuscles and even plague bacilli may be observed. The plague bacillus does not usually occur in kidney tissue in particularly large numbers and it is probable that only when this organism is present in considerable numbers in the capsular space of the glomeruli or where there has occurred haemorrhage in the urinary system will the plague bacillus be found in the urine. In grave cases of plague haematuria is not uncommon and suppression or retention of urine occasionally occurs. Severe uterine haemorrhages may develop and in pregnant women abortion always occurs which is usually fatal to both mother and child.

**Digestive System.**—The mucous membranes of the mouth and throat are more or less hyperaemic and occasional haemorrhagic patches are present. The tonsils may be swollen and hyperaemic and in instances in which infection has occurred through the mucous membrane of the mouth or throat a bubo may form in the tonsil and oedema of the glottis may occur. In these instances as well as in pneumonic plague the sputum contains the plague bacillus. Apart from the haemorrhages which may occur in the mucous membrane of the stomach or intestine the other lesions of the alimentary tract are not of special clinical significance. Vomiting preceded by nausea is a common early symptom of plague sometimes the vomiting persists and then the vomitus is likely to contain blood. Constipation is usual in plague but diarrhoea sometimes occurs and in some cases the stools are dysenteric in character and contain much blood. During the epidemic of primary pneumonic plague in Manchuria several cases of primary intestinal plague were reported in which bloody diarrhoea appeared to be the most prominent symptom. However none of these cases was studied at necropsy and it appears that no definite evidence of the occurrence of primary intestinal infection during the epidemic was produced. In the faeces in which plague bacilli were reported in the faeces infection had evidently occurred secondarily from the blood. Albrecht and Ghon in the report of the Austrian Commission have mentioned the only suggestive case of primary intestinal plague occurring during a bubonic epidemic of plague and even in this case the evidence of such infection is not conclusive. However it seems established that primary intestinal plague has been produced in rats by feeding large quantities of virulent plague bacilli. In many instances during the Manchurian epidemic the patients with pneumonic plague must have swallowed enormous numbers of plague bacilli in the saliva and sputum. Nevertheless in none of the necropsies performed during the epidemic were evidences of primary intestinal infection present nor was serious involvement of the intestine encountered. This fact certainly speaks strongly against the existence of primary intestinal plague in man and would seem to show that even if the intestines are sometimes secondarily involved this condition in human beings must be very rare. It has not been possible



and even scarlet fever. More rarely buboes form in the epitrochlear region or popliteal space the mammary gland testicle or in isolated glands in other parts of the body.

Generally the plague bubo at the onset is hard to the touch and very painful. Often at the time of onset of the bubo pain in it is the symptom of all others of the disease most complained of. In rare instances however the pain may not be marked. Usually if the bubo is in the groin the pain is sufficient so that the patient lies in bed with the thigh flexed and the leg drawn up to relieve any pressure on the inflamed glands while if the bubo is in the axillary region the affected arm is held away from the side. The bubo may terminate by resolution suppuration or induration.

If the bubo suppurates the gland becomes at first more swollen and the overlying skin gradually more inflamed and tense during the first week. Later the gland begins to soften and necrosis then occurs more quickly. Frequently the whole center of the gland breaks down into an abscess cavity and perforation then occurs revealing a cavity with dark scarlet or bright red walls. Later the walls become reddish yellow in appearance and emit whitish yellow pus. On microscopical examination of the pus normal and degenerating plague bacilli are found and many polymorphonuclear leucocytes and degenerating endothelial cells. The bacilli are often seen engulfed in phagocytic cells. In the later stages the buboes often become secondarily infected with other microorganisms particularly the pus cocci. Rarely the bubo does not perforate for several weeks. Sometimes its suppuration is accompanied by much sloughing of the skin in the vicinity when fairly large ulcers result with indurated infiltrated margins. In some instances the lesions may heal in from a week to ten days but with larger buboes sometimes complete cicatrization does not occur for a month or two. In many other cases the bubo terminates by resolution. The tenderness and periglandular infiltration then gradually decrease the overlying and adjacent skin becomes softer and the glands may eventually return almost to their normal size with but moderate induration about them. In other instances an enlarged cicatricial node remains at the site of the bubo.

**Cellulo Cutaneous Plague**—The occurrence of petechiae and of larger ecchymoses in the skin have already been referred to. Plague carbuncles have also been reported. They occur most commonly on the buttocks or back sometimes on the flanks or abdomen the shoulders or posterior surface of the legs and arms. They generally make their appearance in the later stages of the disease and usually originate about ecchymotic patches. Subsequently a vesicle is formed which soon ruptures and reveals a well circumscribed patch which may measure 1 centimeter or more in diameter. The base of the lesion is usually moist and either brownish red or bluish in color while the margins are indurated and infiltrated. The necrosis in some instances becomes deeper and large indolent ulcers are formed (Fig. 170). Sometimes there is considerable oedema about the ulcers and plague bacilli may be found in the oedematous fluid which exudes. However in indolent ulcers degenerating plague bacilli and pus cocci are often found and not infrequently other bacilli. In a small proportion of bubonic plague cases what probably constitutes the primary lesion may be observed. This consists usually of a small vesicle or papule which may become pustular and which is situated on the skin drained by the inflamed lymphatics in the region of the bubo. It perhaps sometimes indicates the original point of the infected flea bite. Microscopical examination of the contents of these lesions frequently shows large numbers of plague bacilli.

**Respiratory System**—In severe cases of bubonic plague oppression of the chest is often complained of. As the disease progresses the breathing becomes labored and the respirations increase in frequency sometimes numbering from 30 to 60 per minute. Cough is frequently present. The sputum may be viscid at first but often becomes purulent and sometimes blood stained. Auscultation and percussion frequently reveal signs of congestion and oedema at the bases of the lungs. Bronchitis is also not uncommon. Pneumonia occurs in plague first as primary plague pneumonia in which the alveoli and sputum contain plague bacilli in enormous numbers. This form has already been thoroughly discussed elsewhere in this article. Secondary bronchial pneumonia also due to the plague bacillus may result metastatically and emboli and abscesses may

also very suggestive of typical plague infection. The reddened and congested eyes and the injection of the conjunctivae together with the flushed bloated and anxious countenance is also often striking. When these symptoms are taken into consideration with the hurried respirations, restlessness and other rapidly developing cerebral symptoms the disease is usually readily recognizable. However in mild cases of plague practically none of these symptoms may be accentuated or even present. The bubo which is the most characteristic symptom of bubonic plague usually appears within 24 hours of the onset but it may not be detectable until the third, fourth or even the fifth day of the disease. In some cases moreover distinct buboes are not found but swollen tender glands are present. When the other symptoms suggest plague a careful search for these inflamed glands should be made particularly in the inguinal axillary or cervical regions. Sometimes a careful examination of the abdomen with deep palpation will reveal a swollen lymphatic in the iliac or lumbar region.

However mild or moderately severe cases of *bubonic plague* with adenitis may be confused sometimes with climatic bubo, venereal bubo, febrile adenitis, filarial infection or certain other diseases. Since the bacteriological examination is a simple procedure and gives reliable information the final diagnosis should always depend upon it and in bubonic plague the bacillus should be sought for in the bubo or swollen lymphatics and in the blood.

In *primary septicaemic plague* there may be no clinical signs from which a diagnosis can be made though the fever in connection with the profound disturbances of the circulatory and nervous systems which result from profound toxæmia may suggest the diagnosis. However the bacteriological diagnosis made from the study of the blood is essential in such cases.

In *primary pneumonic plague* the diagnosis is usually clear from the bacteriological examination of the sputum in which the bacillus is found in enormous numbers and often in almost pure culture.

A rise in temperature and an increased pulse rate are usually the earliest symptoms observable but before the sputum appears the diagnosis may be doubtful. An examination of the blood either microscopically or by culture may reveal the diagnosis since during the great Manchurian epidemic all the cases became septicaemic. The blood should always be examined early by cultural methods as in the primary septicaemic cases involvement of the lungs may not occur. The bacteriological diagnosis is the only certain one for excluding pneumonic infection due to microorganisms other than *Bacillus pestis* but from the general condition of the patient in connection with the absence of marked physical signs in the lungs the diagnosis of pneumonic plague infection is often practically suggested. Latent herpes has not been observed in primary pneumonic plague. The presence of numerous coarse pearly or sibilant bronchial rales in the lungs is an argument against pneumonic plague infection. The sputum in pneumonic plague is not purulent as it frequently is in catarrhal bronchitis or in bronchial pneumonia and it is not so tenacious and has not the rusty appearance of the sputum so often seen in croupous pneumonia. The cough is usually not so painful as in croupous pneumonia. The duration of the disease is usually less than two days though in many cases does not last longer than six to ten hours after the onset of symp-

to isolate the plague bacillus from the faeces in cases of bubonic plague probably some times largely on account of its association with so many other microorganisms though it seems very probable that in those cases in which the plague bacillus is present in the blood during life and extensive intestinal haemorrhage has occurred that it may be present in the bloody evacuations also

**Nervous System**—Pathological anatomical conditions in the nervous system are unusual. Meningitis occurs only occasionally as does haemorrhage of any degree in the brain substance. A few punctate haemorrhages may be more commonly observed at autopsy in the meninges mesencephalon and medulla oblongata. The nervous symptoms, which are often marked are largely dependent upon the toxæmia and congestion and hence are functional.

Particularly striking among these at the onset of the attack are the great depression anxiety and distress depicted by the countenance the severe headache stammering hesitating speech and restlessness. In native patients who frequently come to the hospital for treatment the gait is often staggering due to lack of mental concentration and giddiness. There is no paralysis of the limbs but the voluntary muscles are evidently not completely under the control of the individual. Usually as the disease progresses the toxæmia affects the intellect to an even more marked degree and mental dullness drowsiness confusion of ideas and delirium either acute or of a low muttering type are common symptoms. Some patients however remain in a semicomatose condition from which they may be easily aroused and will answer questions slowly and with difficulty. Others however lie in a state of stupor from which it is impossible to arouse them. Rarely there may be complete aphasia. Delirium when it occurs may be either noisy or acute but the acute forms are more frequent. Sometimes it is necessary to put the patient under restraint in bed to keep him from escaping or injuring himself. In the later stages of the disease tremors twitchings and spasms particularly of the muscles of the face neck and extremities with sometimes stronger convulsive seizures are occasionally observed. In other instances the toxæmia manifests itself in the production of an apathetic condition the patient lying in a state of stupor with the mouth partially opened and the eyes either wide open or partially closed glass like and with a vacant expression. Usually during the height of the disease the patients are unable to sleep except for short periods. In rare instances a state of dementia aphasia or even ataxia may remain for a temporary period during convalescence but usually the nervous disturbances disappear rapidly during convalescence.

## DIAGNOSIS

**Clinical Diagnosis**—The early diagnosis of plague is obviously of very great importance not only on account of the serious nature of the presence of the disease in a household or community, but also with reference to the serum treatment for on account of the special anti-infectious nature of plague immune serum this serum is only effective in bubonic plague when given in the early stages of the attack.

The occurrence of high fever of sudden onset with adenitis and prostration, and other evidences of marked toxæmia and affection of the nervous system is very suggestive of plague. Perhaps in no other disease does the heart weakness manifest itself so early and severely as it does in virulent plague infection and the very rapid feeble pulse may be present even from the beginning of the attack. The severe and early affection of the nervous system as evidenced by the headache halting speech mental confusion dizziness staggering gait and great prostration is

In thirty-one cases of bubonic plague which resulted fatally and were studied in Manila by Calvert bacilli were found in the blood 24 hours before death in 100 per cent 48 hours before death in 48.39 per cent 72 hours before death in 25.8 per cent 96 hours before death in 9.68 120 hours before death in 3.22 per cent. The organisms were very scanty in the longer periods before death.

Microscopical examinations of the hardened stained preparation (as above described) of the blood may also be made. Some of the preparations should also be made by placing thick drops of blood upon the slide and after drying they may be washed in distilled water to free the preparation from haemoglobin before staining. In primary pneumonic and other forms of severe septicaemic plague the bacillus may sometimes be detected in this manner since the bacilli are sometimes as numerous in the blood as they are in anthrax in animals. Nevertheless in the very early stages of pneumonic plague as in bubonic plague cultures from the blood are frequently negative. In pneumonic plague the organism may generally be cultivated from the blood from twenty-four to forty-eight hours before death and practically always from the blood a few hours before death.

In a small percentage of bubonic plague cases a primary vesicle of infection may be found on the skin. After careful sterilization of the surface of such a lesion some of the fluid should be withdrawn in a sterile syringe and cultures and inoculations of guinea pigs and microscopical preparations in the manner described above should be made from the fluid. These vesicles often contain plague bacilli in large numbers. If attempts are made to obtain the plague bacillus by culture from the urine it should be borne in mind that the plague bacillus develops very slowly in cultures made from the urine and that such cultures frequently result negatively.

*The Sputum*.—In pneumonic plague the sputum should be examined both microscopically and culturally for the plague bacillus and plate cultures should also always be prepared from the sputum. Although usually in pneumonic plague the sputum contains enormous numbers of plague bacilli sometimes in bubonic plague complicated with secondary plague pneumonia there may be very few or no plague bacilli present. In such instances in which the organism cannot be found microscopically however it may be detected by cultural animal inoculation. In the sputum the plague bacillus may be mistaken sometimes for *Diplococcus pneumoniae*. Bipolar staining organisms other than the plague bacillus have been sometimes found in the sputum. While in the microscopical examination of the sputum Gram's stain is of valuable aid in arriving at a diagnosis nevertheless Gram-negative bacilli have been occasionally found in the sputum which proved later not to be plague bacilli. Usually however if the sputum is blood stained from the microscopical examination with the aid of Gram's stain there is no difficulty in arriving at a diagnosis since the plague organism is generally present in such large numbers. In the later stages of pneumonic plague involution forms are frequently found in great abundance in the sputum. In doubtful cases the sputum may be inoculated into guinea pigs by the cutaneous method as described below. This is practically an infallible method of detection of their presence. Finally for diagnosis of a fatal case of pneumonic plague where an autopsy is not permitted lung puncture with a syringe will usually furnish material with which a definite diagnosis can be made. *Bacillus pestis* being present in enormous numbers in the microscopical preparations made with the prepared material.

Notwithstanding some earlier published statements to the contrary the bacillus of primary pneumonic plague has been shown to be morphologically culturally and by animal inoculations identical with the most virulent strains isolated from bubonic plague cases. Only with reference to virulence then may there sometimes be variation. During epidemics of bubonic plague strains of different virulence may be encountered. Thus the organisms isolated from cases of *pestis minor* are usually of lower virulence. Since infection of man in bubonic plague is acquired through rats and as different rats of different susceptibility may harbor strains of plague bacilli at times of more or less virulence so obviously these strains isolated from different human cases of plague will vary in virulence. In primary pneumonic plague however infection being transmitted directly from man to man and the lung constituting a very

toms Cases sometimes survive for three and more rarely for four days but not over one week

**Bacteriological Diagnosis**—*Bacillus pestis* occurs in the invaded lymphatic glands, the blood and the sputum Its presence in the urine is too inconstant to render the bacteriological examination of the urine of any great value in diagnosis

**Buboes**—For obtaining material for bacteriological examination from the inflamed lymphatic glands or *bubo* a ten cubic centimeter syringe with an eighteen gauge needle is advisable as considerable suction is desirable in order to aspirate successfully sufficient fluid from the gland

After the syringe needle has been sterilized preferably by dry heat the skin over the gland is cleansed with soap and water and alcohol and then painted with tincture of iodine After the iodine has dried the gland is held with the left hand and the needle attached to the syringe inserted well into the substance of the gland and moved up and down a few times in the gland substance while aspiration is being performed After aspiration the skin about the point of puncture should be disinfected preferably with bichlorid solution 1:1000 or absolute alcohol At least several drops of fluid may be obtained from aspiration of the gland in this way The fluid in the syringe should then be ejected into a small sterile test tube If the bacteriological diagnosis is to be conducted at once a drop of the aspirated fluid is then well smeared with the bacteriological platinum loop over the surface of an agar slant culture and the needle then passed (without re-inoculation) over the surface successively of a second third fourth and fifth agar slant culture On the surface of the media of some of these tubes isolated colonies of the plague bacillus will develop within twenty four to forty eight hours when this organism is present A second and third drop of the fluid from the bubo should then be spread with the platinum loop on each of two microscopic slides the preparation being rubbed with the loop until thoroughly dry After these preparations have been hardened either in the flame or preferably by placing a few drops of absolute methyl alcohol upon them for one or two minutes one of the slides should be stained with Loeffler's methylene blue or with Giemsa's stain and the other by Gram's stain With the remaining fluid which was aspirated from the gland a guinea pig should be inoculated as described below

In all these procedures it should be kept in mind that one is working with highly infectious material and every precaution must be taken not to infect ones self or others or any objects about The fluid from the syringe must be expelled cautiously lest some of the plague bacilli be vaporized into the surrounding atmosphere since if these organisms were inspired pneumonic plague would almost certainly result

**Blood**—For the bacteriological examination of the blood in plague a ten cubic centimeter syringe sterilized in dry heat may be advantageously employed After a thorough cleansing of the skin of the arm at the bend of the elbow with soap and water and alcohol and by painting with tincture of iodine the needle should be thrust into one of the veins in this locality and from five to ten cubic centimeters of the blood withdrawn A bandage placed around the upper portion of the arm before the puncture is made will aid in distending the vein From one to two cubic centimeters of the blood may be injected directly into melted agar tubes which are then poured onto Petri dishes or from five to ten drops placed on agar slant cultures Five cubic centimeters of the blood may also be inoculated directly into a one hundred cubic centimeter flask of bouillon (or a smaller amount in a tube of this media) from which other agar plates or slant cultures may be made later on arrival at the laboratory where a guinea pig may be also inoculated subcutaneously with from one to two cubic centimeters of the blood If the blood has to be carried for some distance from the patient to the laboratory then it is preferable to place in the syringe before aspirating the blood to prevent its clotting one or two cubic centimeters of a five per cent citrate of soda solution which has been carefully sterilized

*pestis* is permanently in the rough phase and that it possesses no smooth somatic antigen. However this idea would appear to require further confirmation.

There are also difficulties in the preparation of a satisfactory plague immune agglutinating serum. Such a serum may only be obtained after many repeated inoculations of an animal and during the period of immunization it frequently dies from toxæmia. Many of the plague immune serums that are employed for treatment possess practically no agglutinating power against the plague bacillus. Hence if one employs this test clinically all these points must be borne in mind in performing the agglutination test and a large number of control tubes should always be made some prepared with normal serum as well as others prepared with the plague immune serum. In performing the test for the diagnosis of the organism a serum agglutinating in dilutions of at least 1:1000 is recommended. Only an organism very closely related to the plague bacillus such as the *Pasteurella pseudotuberculosis* bacillus will agglutinate with a satisfactory plague immune agglutinating serum.

	<i>Past pestis</i>	<i>Past pseudotuberculosis</i>	<i>Past a septica</i>
Motility in 18 hour cultures at 22 C	—	+	—
Litmus milk	— or slight acid	Alkaline	—
Sugars	Acid in glucose, maltose, mannitol and salicin	Acid in glucose, maltose, mannitol and salicin some times in sucrose	Acid in glucose, mannitol and sucrose sometimes in maltose
Indole	—	—	+
M.R.	+	+	—
Methylene Blue reduction <sup>1</sup>	—	+	+
Growth on MacConkey	+	+	—
Pathogenicity to white rats	+	—	+

Observations on a fairly few strains

Kauffmann (1932) has obtained evidence that *Pasteurella pseudotuberculosis* has more complex antigenic structure than *Past pestis*. *Pseudotuberculosis* possesses 3 antigens: (1) a flagellar antigen, (2) a smooth somatic antigen of which there are at least five types, (3) a rough somatic antigen which like the flagellar antigen is common to all strains. The rough somatic antigen is apparently identical with the somatic antigen of the plague bacillus and it is to this antigen that the two organisms owe their affinity.

Like the smooth somatic antigen of *Past pseudotuberculosis* is related to the O antigen present in related organisms of the *Shigella* group. Since *Pasteurella pestis* is non-motile it does not exhibit the floccular type of agglutination characteristics of the flagellated species and agglutinates rather in the form of very small clumps. Nevertheless the difference at once of *Past pestis* and *Past pseudotuberculosis* may be very definitely. *Past pseudotuberculosis* is often motile in broth culture incubated for 8 hours at 20–22 C whereas *Past pestis* is uniformly non-motile. The production of alkali in litmus milk by *Past pseudotuberculosis* and usually the production of acid in this medium by *Bacillus pestis* may be noted. The growth of *Past pseudotuberculosis* is usually much more rapid and luxuriant. *Past pseudotuberculosis* is also comparatively harmless for white rats in which animals *Bacillus pestis* causes fatal infection. The agglutination test for differentiation is decidedly less satisfactory.

Bessonova and her associates (1937) have reported that out of 214 strains of the plague organism five underwent spontaneous transmutation to *Past pseudotuberculosis*.

favorable culture medium for the development of the organism the plague bacillus isolated from these cases is of maximum virulence

The bacteriological diagnosis of the plague bacillus is based upon the morphological and staining properties of the organism its cultural characteristics, and the result of animal inoculation. The agglutination and other serum tests are not of such assistance in identifying the plague bacillus as they are with a number of other microorganisms

*Morphology*—In the hardened preparation the noteworthy characteristics of the plague organism are the bipolar staining the decolorization by Gram's stain and polymorphism (coccoid bacillary and involution forms). In the stained microscopic preparations made from the buboes and organs of plague cases the most characteristic form is a short bacillus more or less ovoid swollen in the center and rounded at the ends and usually exhibiting marked bipolar staining the central portion either remaining uncolored or staining lightly. No flagella are visible and there is no motility. In cultures the formation of involution forms upon 3 per cent salt agar is noteworthy. However the diagnosis cannot be made from the examination of the microscopical preparations alone since morphologically it may be confused with the different organisms of the haemorrhagic septicaemia group or of the Gäertner paratyphus group or sometimes with *Bacillus mucosus capsulatus*. Culturally the most characteristic feature of the plague organism is the appearance of the delicate transparent and dewdrop-like colonies which appear on agar after 4 to 48 hours.

*Animal inoculation* is important for the diagnosis and the guinea pig is by far the most satisfactory animal since a single virulent plague bacillus may cause fatal infection in this animal. The inoculation of the guinea pig should generally be made with the suspected material by the cutaneous method and this is a necessity if the material is badly contaminated with other microorganisms. However in the case of inoculating blood the subcutaneous method should be employed. In performing the cutaneous inoculation the skin of the lower abdomen over a small area should be shaved and then with a scalpel the suspected material should be lightly rubbed in and the skin lightly scarified with the point of the knife. If the virulent plague bacillus is present in the suspected material the animal will die usually within 3 to 5 days with the characteristic lesions described on p. 665.

The *agglutination test* is not of great clinical importance in the diagnosis of plague. Owing to the fact that the plague organism forms on agar a mucus like substance it is very difficult to obtain satisfactory suspensions of it in normal saline solution as in spite of all care in making such suspensions one frequently finds that the larger particles of bacteria have settled to the bottom of the tube and form a precipitate so that when the reaction is employed with a plague immune serum pseudoagglutination reactions may be obtained in this manner. Numerous studies have been made for the purpose of obtaining more satisfactory suspensions of the plague bacillus for agglutination tests in which the organisms would not undergo spontaneous sedimentation. It has been shown that the plague bacillus forms more mucus and envelope antigen when cultivated at 37 C than when grown at 32 C hence it is sometimes of advantage to employ cultures for this test which have been grown at the lower temperatures. The employment of 0.5 per cent sodium chloride solution is also recommended in place of 0.85 per cent for making the suspensions of the plague organism. Finally since spontaneous flocculation is not as likely to occur in freshly isolated strains of the plague bacillus as in those which have been preserved for long periods of time on agar if agglutination tests are to be made with a culture of the plague organism it is recommended that it first be passed through an animal unless it has been very recently isolated from a case of plague.

The occurrence of smooth and rough colonies has already been discussed. According to Schutze there are two antigens in the plague bacillus one corresponding to the envelope and the other to the s-matic substance. Schutze believes that *Pasteurella*

In *dengue fever* while there is a sudden onset with high fever and headache and there may be occasional glandular enlargements the pain about the joints and muscular insertions the erythematous rash leukopenia and reduction of the polymorphonuclear leucocytes should give a very early diagnosis.

In plague even after the bubo has appeared difficulty may arise in diagnosis between *disinalis* and *teneris* buboes or buboes secondary to other infections and in cases with lung symptoms between pneumonic plague and pneumonia of other origin. *Climatic bubo* (Lymphogranuloma inguinale) may particularly be confused with mild cases of bubonic plague. It occurs particularly in sailors. The inguinal glands are usually affected. The incubation period is usually long and the onset gradual. The swollen glands are generally only slightly tender. Fever is usually not high and the gland rarely suppurates or does so only after a long period. Gland puncture reveals no microorganisms either on microscopical examination or on culture the disease being caused by a filterable virus. The Frei-Hoffman intradermal reaction may be obtained in climatic bubo.

In venereal bubo either there will be ulcerations on the genital organs or a former history of such. In a certain percentage of these cases a pure culture of the bacillus of Ducrey may be found in material obtained from the bubo by puncture. However sometimes other bacteria or pus cocci are also present. The bacillus of Ducrey stains fairly well with anilin dyes but loses color rapidly when a decolorizing agent like alcohol is applied. Cultures of this organism may be frequently obtained upon blood agar but not on ordinary media and inoculation on guinea pigs with it gives negative results. In bubo resulting as secondary infection from ulcerations or wound of the skin there is more or less lymphangitis which is rare in plague and the infecting organisms will be found in the gland by microscopical examination or in culture or the cultures from the glands will be sterile.

In all doubtful cases the bacteriological methods already described in connection with the buboes should be employed and special microscopical examinations of the blood and in cases with lung symptoms of the sputum should be made for animal parasites or bacteria in order to exclude other infectious diseases.

It is also important that an autopsy should be performed for diagnostic purposes on all fatal cases that have been in any way suspicious of plague. The general venous engorgement haemorrhages in the different organs cloudy or fatty liver enlarged heart spleen swollen lymphatic glands and the presence of a bipolar staining bacillus in the heart's blood and organs which upon isolation by culture and inoculation into guinea pigs causes the typical lesions of plague will certainly reveal the correct diagnosis.

During the last great epidemic of influenza several writers who were evidently not familiar with the clinical features and pathology of primary pneumonic plague or the literature upon this subject reported that there was some confusion in the differentiation of influenza and primary plague pneumonia. While patients with severe influenza may show great prostration and cardiac weakness such as is observed in plague the onset of influenzal pneumonia and the clinical course of the disease are quite different from primary pneumonic plague. In influenzal pneumonia the onset of the disease occurs with symptoms of ordinary influenza and the pneumonia is a secondary complication superimposed on the primary disease. In primary plague pneumonia the pneumonia occurs at the onset as a primary infection and the course is much more acute. Moreover the bacteriology of the two diseases is entirely distinct and by the bacteriological examination these two infections can be certainly differentiated. As a matter of fact neither in the United States nor in Europe did the recent epidemic of influenza sufficiently resemble primary pneumonic plague as to cause confusion of diagnosis between the two to those familiar with both diseases.

### PROGNOSIS

Plague is the most fatal of all epidemic diseases. In primary pneumonic and septicaemic plague the prognosis is absolutely unfavorable many stating that every such case dies. As regards bubonic plague the



and came to differ entirely from S and R variants of *Past pestis*. The transmitted strains have remained stable for as long as 7 years.

The other haemorrhagic septicaemia bacilli can often be differentiated from *Past pestis* by their fermentation of sucrose, their production of indole and their negative methyl red reaction. However sucrose is fermented by certain strains of *Past pseudotuberculosis*. Topley and Wilson (1936) give the foregoing table of differentiation.

They suggest that the different strains of the organisms of the haemorrhagic septicaemia group might be included under the specific name of *Pasteurella septica*.

**Agglutinins in the Patient's Serum**—The agglutination test has obviously from a theoretical standpoint, not only the advantage of identification of the organism cultivated from a suspected case but also that of the diagnosis of the disease by the demonstration of antibodies in the patient's serum. While this reaction may occasionally be important in plague for the diagnosis of the organism, the reaction is of very little value for the diagnosis of the disease. It is true that agglutinins for the plague bacillus may develop in the blood serum of patients recovering from plague and sometimes they are present even in the late stages of the disease but their amount is always small. The reaction rarely occurs in dilutions of the serum higher than 1:5 or 1:10 and in many undoubted cases of plague no reaction is in fact obtained.

In pneumonic plague, the agglutination test has no clinical value whatever for the patients succumb to the disease before antibodies are produced in quantities that are capable of detection. For the same reasons the other serum reactions such as complement deflection test and the bacteriolytic reaction of the serum have also practically no clinical value in the diagnosis of plague.

### DIFFERENTIAL DIAGNOSIS

In the early stages of the disease plague must be differentiated from certain other fevers which may occur in the tropics such as typhus, relapsing fever, malaria, dengue fever and even typhoid. If there is no suspicion of plague and no bacteriological examination made the clinical features and occasionally even the postmortem appearances may be attributed to some other disease.

Severe typhus fever may to the uninitiated sometimes resemble plague. The onset of typhus which is frequently with fever, headache, chill, pain in the back and limbs, injected conjunctivae, mental disturbances, rapid pulse and prostration may sometimes cause confusion. However the eruption in typhus which appears by the fourth day is different from the petechial and other haemorrhagic lesions of the skin observed in plague and it is much more extensive and pronounced than is seen generally in a case of plague. In typhus fever the eruption at first usually consists of discrete sharply defined pink macules from about 2 to 5 mm in diameter with round or irregular margins which disappear on pressure. It is general in character except on the face. Although in the later stages the rash frequently becomes red and even purplish red and then may not disappear on pressure it does not assume as marked a haemorrhagic character as occurs in plague. Also the leucocyte count in typhus is more commonly below 18,000. The agglutination test performed with *Bacillus proteus*  $\alpha$  which is positive in typhus in approximately 95 per cent of the cases may often assist in further differentiating typhus from plague.

Malaria and relapsing fever can of course be at once differentiated by the blood examination and by finding the specific protozoan parasites for these diseases as well as by their subsequent clinical course.

In *dengue fever* while there is a sudden onset with high fever and headache and there may be occasional glandular enlargements the pain about the joints and muscular sections the erythematous rash leukopenia and reduction of the polymorphonuclear leucocytes should give a very early diagnosis.

In plague even after the bubo has appeared difficulty may arise in diagnosis between *climac* and *venereal buboes* or buboes secondary to other infections and in cases with lung symptoms between pneumonic plague and pneumonia of other origin. *Climac bubo* (Lymphogranuloma inguinale) may particularly be confused with mild cases of bubonic plague. It occurs particularly in sailors. The inguinal glands are usually affected. The incubation period is usually long and the onset gradual. The swollen glands are generally only slightly tender. Fever is usually not high and the gland rarely suppurates or does so only after a long period. Gland puncture reveals no microorganisms either on microscopical examination or on culture the disease being caused by a filterable virus. The Frei-Hoffman intradermal reaction may be obtained in climatic bubo.

In venereal bubo either there will be ulcerations on the genital organs or a former history of such. In a certain percentage of these cases a pure culture of the bacillus of Ducrey may be found in material obtained from the bubo by puncture. However sometimes other bacteria or pus cocci are also present. The bacillus of Ducrey stains fairly well with anilin dyes but loses color rapidly when a decolorizing agent like alcohol is applied. Cultures of this organism may be frequently obtained upon blood agar but not on ordinary media and inoculation of guinea pigs with it gives negative results. In buboes resulting as secondary infection from ulcerations or wound of the skin there is more or less lymphangitis which is rare in plague and the infecting organism will be found in the gland by microscopical examination or in culture or the cultures from the glands will be sterile.

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infection. These examinations are sometimes of very great importance. Plague rats were found in New Orleans two years before the epidemic of human plague occurred. Our Public Health Service has recommended the examination of 1000 rats per 10 000 human population as affording reliable evidence of plague infection among rodents of a community.

The early detection and diagnosis of human cases of the disease are not only important in prevention but also in regard to treatment. All deaths during an epidemic no matter from what cause must be investigated and autopsies should be performed and bacteriological examinations made. Cases of the disease should be isolated and their clothing disinfected of any fleas under proper precautions and the usual disinfection of their excreta and surroundings exercised. The search for patients by house to house inspection is a very important measure since a large number of plague cases are usually concealed during epidemics by their relatives and friends. Ordinances should of course be passed compelling the report of any suspected case. If infected plague cases are found and the construction of the house permits there should be a preliminary disinfection with sulphur dioxide, methyl bromide or other substances that may be depended upon to kill rats and fleas and a search made in the neighborhood for secondary cases both in man and rodents. Contaminated objects in and about houses may be disinfected with 1:1000 bichloride of mercury, 2½ per cent carbolic acid, 10 per cent formalin or 1 per cent solution of chlorinated lime. In places where plague is endemic or likely to become epidemic there should be a special hospital as well as a special diagnostic laboratory. Provision must be made for the isolation of human cases upon their arrival until they have been divested of their clothing and disinfested of any fleas. All of the clothing should be immediately placed in a bag and disinfected in a steam sterilizing chamber. Attendants who handle patients on their arrival or their infected clothing should wear gloves and special uniforms designed to prevent the entrance of fleas. High boots are particularly desirable. The hospital itself must be well screened and protected from insects and should be rat free. Obviously particular attention must be paid to the exclusion of fleas in countries where these insects are common. Fabrics and other objects which become contaminated with the discharges should be thoroughly disinfected by proper methods. Cremation of dead plague bodies should be recommended. Protective inoculation should also be advised particularly for attendants and persons about the hospitals and for those who are performing or assisting at autopsies upon plague cases. During bubonic plague epidemics the plague hospital provided it is free from rats and fleas presents no particular dangers for attendants.

Rat and flea extermination has been one of the important prophylactic procedures but it is often difficult to accomplish in some localities. However in others the building of rat proof areas has gradually abolished plague. Hence in regions where plague exists an extensive campaign should be undertaken against rats and all buildings which are constructed so as to permit of the abode of rats should be gradually rebuilt in the infected districts.

mortality averages 75 per cent in India but frequently varies from 60 to 90 per cent. In the Egyptian epidemic of 1900 there was an average mortality of 50 per cent. The mortality in natives is generally much higher than among Europeans, the latter often showing death rates under 25 per cent, while in the same epidemic natives may show a mortality of from 75 to 95 per cent. In South America, the disease is usually milder and the mortality may be only about one third of that given in India and China. Barreto reports a mortality in Brazil of 35-43%. In the recent small European outbreaks the mortality has also been low. Plague pneumonia, however, is fatal for Europeans as well as natives.

### PROPHYLAXIS

**General Prophylaxis of Bubonic Plague**—In the prevention of bubonic plague the public health campaign must center upon the early discovery of cases of the disease, their isolation from rats and fleas and the destruction of rats and fleas in infected areas. In other words, bubonic plague is not usually spread by direct contact with the sick. However, rats and rat fleas may be carried from one place to another by ships, railways or other methods of communication.

Plague being primarily an infection of rodents and transmitted commonly to man from such rodents by infected fleas, general prophylaxis in bubonic plague consists primarily in the prevention of contact between man and such infected rodents and fleas and hence in the general destruction of rats and fleas in regions where plague exists or is likely to exist. Since when rats are reduced in number there is more likelihood that rat fleas will seek the body of man for food, it is well to employ when possible measures that will destroy simultaneously both rats and fleas. It has been noted that a high death rate among rats as the result of a plague epizootic may act as a factor in an outbreak of human plague. The elimination of human fleas in areas where plague infection is present is also very important.

One preparation known as pesterine, which consists of kerosene 10 parts, soft soap 1 part and water 5 parts (the soap being dissolved in the water and the oil being gradually stirred into the hot mixture) is often recommended as a flea insecticide. Sulphurated hydrogen 1 per cent solution is also a good pulicide. A 5 per cent solution of combined creosol to which naphthalene has been added is likewise of value for the destruction of fleas.

It must be realized that not only the infected rodent but also the human plague patient constitutes a focus of infection and that hence prophylactic measures against plague must include an early diagnosis and detection of cases of human as well as of rodent plague. For this purpose special bacteriological laboratories which permit of thorough isolation and disinfection should be established and equipped with special animal cages and apparatus for the study and diagnosis of plague. In places where plague is endemic it is advisable to collect periodically and make examination of rats, since human plague outbreaks are frequently preceded by rodent

each containing 3 grains of barium carbonate. The baits should be fairly fresh as a stale one is very rarely eaten by a rat. Red squill is especially recommended in the United States Army.

*Vaccines* have been recommended for the wholesale destruction of rodents. These are usually either cultures of the *B. typhi murium* type or the paratyphoid B type which is frequently the cause of meat poisoning in man, or of the *B. enteridis* or Gärtner type which has been associated with gastrointestinal disturbances. The so-called Danysz virus usually *B. typhi murium* is pathogenic usually for rats under laboratory conditions but has feeble powers of propagating itself from rat to rat under natural conditions. It rapidly loses virulence when exposed to light and air. The use of these viruses is not recommended for the general destruction of rats since they have usually proved to be inefficient for this purpose and moreover they are not absolutely harmless to man and instances of sickness and death in human beings from infection by them have been reported.

In South Africa attempts have been made to destroy gerbilles (one of the chief carriers of plague infection) by another virus *Listerell monocytogenes*. This organism is very fatal to gerbilles by ingestion but is not virulent for most other rodents. The use of the virus has been tried over a gerbille infected belt of country some 20 miles long. The disease it causes in the gerbille is known as the Tiger River disease and the prepared culture as the Tiger River virus. Its actual value has not yet been demonstrated.

**Fumigation for Rodents and Fleas**—In the case of the occurrence of plague on board ship or the arrival of a ship from a plague infected port fumigation of the ship should be practiced. Grubbs also emphasizes the importance of the fumigation of cargo in lighters in plague infected ports. Hydrocyanic acid gas is undoubtedly the most efficient destroyer of both rats and fleas but it is very dangerous and a number of fatalities have been reported in connection with its use. The gas developed from  $\frac{1}{2}$  ounce of KCN to a space of 100 cubic feet acting for 4 hours has generally been regarded as efficient for disinfection. Stitt points out that the great danger from the use of this gas in holds of ships is that it tends to collect in detached spaces or pockets and remains after ventilation of the hold so that persons entering such spaces suffer the poisonous effects of the gas. While sulphur dioxide is less efficient it is on the whole the best suited for general use in plague fumigation. Two pounds of roll sulphur for each 1000 cubic feet of space is regarded as sufficient. The Clayton Gas Apparatus in which the sulphur dioxide is under pressure gives the best results in sulphur fumigation. Carbon monoxide and carbon dioxide and flue or funnel gases from steamers have been recommended for plague prevention work but they are not so satisfactory for while they will kill rats the fleas are often not destroyed and escape.

In the case of ships which have touched ports where plague is present precautions against the transfer of rats from ship to land or from the ships to lighters and the docks to ships when vessels are in port are very essential. There is not much danger of rats going aboard a ship lying out from the dock. It is when a ship goes along side a dock that rats especially go aboard. All boats should be kept at least 4 feet away from the docks and all hawsers should be provided with rat guards. The rat guarding of ships is a matter of very considerable importance. Extremely efficient and practical rat guards for ships' lines have been made of

One of the important measures in rat extermination in rural districts is the regulation of the disposal of garbage. It is most important that only cans with securely fitting tops be used so that rats cannot secure any food from the contents of the can. Precautions should be taken to protect stores of grain and cereals. No particle of food should be left accessible to the rat except baits. Unless the ordinary food supply of the rat is denied the animal it will not eat poisoned food or bait in traps. Rats are not only carnivorous but will eat any kind of cereal or vegetable and in addition they are cannibals.

It has been demonstrated that it is better to build areas rat proof than to try to keep them rat free. The U S Public Health Reports give full information regarding rat proofing in this country. The sewerage should be improved and all filth burned. The separation of the rat from his food supply and the prevention of his entry into human habitation by rat proofing through the use of concrete screening with wire netting and by other barriers and by the use of traps and poisons are all important.

In rural districts in a plague outbreak especial attention should be directed to flooring in stables under surfaces of board walks sealed in attics of houses wharves and sewers. Where sewers have catch basins at street openings the rat has a means of egress from the sewer. These sedimenting catch basins also serve as a breeding place for mosquitoes. It has been estimated that a sewer rat can jump 2 feet but not 3 feet. In rat proofing houses double walls should be eliminated and houses raised well from the ground—at least 18 inches. In plugging up rat holes with concrete broken glass should be added to the concrete. Sheets of galvanized iron driven down several feet have been used as a protecting barrier around grain elevators or ware houses. Concrete is the most satisfactory material to use in rat proofing.

The most satisfactory trap has proved to be a wire spring or snap trap. This type has been shown to be much more efficient than the wire cage trap. All the rats caught should be sent to the bacteriological laboratory preferably in closed containers or canvas bags and after their fleas are killed by chloroform they should be examined and records kept concerning the location where the infected rats were caught.

For the detection of plague infected rats during an epidemic the plan carried out by Carter and subsequently by Heiser in Manila and which proved effective was as follows:

A list of places in which the plague infected rats were found was made. Each was regarded as a center of infection. Radiating lines usually 5 in number were prolonged from this center evenly placed like the spokes of a wheel. Rats were caught along these lines and examined. Plague rats were seldom found more than a few blocks away. The furthestmost points at which the infected rats were found were then connected with lines on a map. The area enclosed by these lines was regarded as a section of infection. The entire rat-catching force was then concentrated along the border of the infected section. They then commenced to move toward the center catching the rats as they closed in. Behind them rat proofing was carried out. One section after another was treated in this way until they had all been wiped out.

**Rat Poisons.**—In districts where rat proofing is not feasible other means such as trapping and poisoning and fumigation may be resorted to. With reference to rat poisons it is important to call attention to the fact that rats will often not eat bread and food which has been particularly handled by human beings and therefore the people who handle or cut the bread or food before dipping it into the rat poison should either wear gloves or have their hands smeared with oil of aniseed or some other similar substance and the board on which the food is cut should be treated in the manner. A very effective poison against rats consists of a phosphorus paste into which the food is dipped. The phosphorus is mixed with glucose in the proportion of 1 to 4 and a fatty base such as lard is employed to prevent spontaneous combustion. Barium carbonate constitutes a very efficient rat poison and a relatively safe one in regard to children and domestic animal. One pound of barium carbonate is mixed thoroughly with 3 pounds of flour or other ground grain in an enamel basin. Sufficient water is added to make the whole into a fairly firm paste. The resulting mass is sufficient for some 2,300 baits.

**Personal Prophylaxis in Bubonic Plague** — This depends upon avoiding plague infected districts contact with plague patients and protection from fleas. People who live under hygienic conditions rarely contract bubonic plague. Nurses and other attendants on the sick ought carefully to seal up and cover any wounds about the hands no matter how trifling. The excreta and bed linen of the patient must be carefully handled and sterilized. For those who are compelled to enter and work in plague infected districts special precautions must be taken against fleas. High boots with the openings at the top around the trousers closed by elastic or adhesive strapping are advisable. Flea proof suits are also recommended. The use of insecticides such as kerosene or crude naphthalene are sometimes of service in repelling fleas. Prophylactic inoculation has also been advised during epidemics of bubonic plague. As soon as definite symptoms of plague appear in those who have been exposed to infection plague immune serum may be injected.

**Protective Inoculation** — A number of different methods of protective inoculation against plague have been described. Haffkine first recommended killed bouillon cultures. Killed agar cultures killed sensitized agar cultures (with serum) extracts of the plague bacillus and living thoroughly avirulent cultures (true plague vaccines) have also been employed. After extensive experimental work the writer 1907 demonstrated that there is little doubt that a higher immunity against plague infection may be obtained from the use of the living avirulent cultures than from the killed organisms and in fact while it is possible to immunize a high percentage of guinea pigs with living avirulent cultures guinea pigs cannot be immunized against virulent plague infection with killed cultures. However it was still maintained by some that it was possible to immunize with killed cultures of the plague bacillus and that they were equally valuable or even better than living avirulent cultures.

More recently Otten 1934-41 in the Dutch Indies Pirie and Grasset 1938 in Johannesburg and Girard and Robic 1938 in Madagascar have also conclusively demonstrated the superior value of living avirulent cultures over dead cultures of *P. pestis* in immunization.

However while this method of inoculation of living avirulent cultures may be the best for some groups of individuals where the preparation of the vaccine can be carefully controlled nevertheless it is probably not a method that can be generally recommended for large numbers of people during a widespread epidemic. When the prophylactic has to be prepared in exceedingly large amounts in the laboratory only a method of employment in which the vaccine is fully sterilized is advisable. The use of the killed bouillon or agar cultures of the plague bacillus unsensitized on account of ease in preparation is today generally employed for prophylactic inoculation against plague.

In the prophylaxis of plague in man in India Haffkine's vaccine has been chiefly used. Formerly broth cultures were grown for 6 weeks at room temperature and heated for  $\frac{1}{2}$  hour at 65°C and 0.5 per cent phenol then added. Now the prophylactic is prepared from a 4 weeks



galvanized iron. These guards will fit on all lines accurately and have straps which hold them perpendicular to the line.

**Quarantine**—Care must be taken also to see that no cases of plague land from ships and particularly that mild cases such as those of *pestis minor*, are not overlooked. Passengers and crews from plague infected ports should be carefully inspected. The temperature of each person should be taken and it is desirable to make special examination for buboes. If a case of suspected pneumonic plague should be found, it should at once be isolated in the hospital and the individuals in contact with it should also be isolated in separate compartments. The employment of an efficient immune serum if available for the contacts should be considered. If a case of bubonic plague is discovered it should also be taken to the hospital but individual isolation is not so necessary for other passengers. It is advisable for vessels which are constantly trading with plague infected ports to have the crew given prophylactic inoculation against plague. The period of detention of the personnel for a plague infected ship has varied from 7 to 10 days.

Maritime transmission of plague is dependent very largely on the escape of infected rodents from ships and the transfer of their infected fleas to rats on the shore. The survey of the U. S. Public Health Service (1937) of ships at Atlantic ports has shown that only 8.4 per cent of the vessels were infested with rats whereas between 1925 and 1927 the number of vessels infested was 50 per cent. Factors which have led to this improved condition have been effective fumigation, rat proofing of vessels, extensive inspection and international certification. Vessels which are free of rats qualify for a reduction of quarantine delays and port dues.

**Disinfection of Houses**—Houses in which one or more cases of plague have developed or in which infected rodents have been found should always be disinfected to kill rodents and insects. After disinfection of houses or rooms several guinea pigs may be placed in them for a few days before human occupation is allowed. If many infected fleas are still present, the animals will often contract the disease. The guinea pig may be successfully infected with a single virulent plague microorganism. Sokhey and his associates (1939) have made an extensive study of the question of the disinfection of houses in Bombay. They have especially studied the new cyanide compounds which are very stable and which give out KCN only when blown into the air in dust, hence they state they are relatively safe and easy to apply. Three chemical preparations, cyanogas, calcid and cymag, have been especially satisfactory. Both in artificial burrows and in houses the rats and fleas exposed were found to be killed, the preparation known as calcid being by far the most effective for this purpose. Stewart and Mackie (1938) have found in the western United States that liquid methyl bromide is very effective in destroying rodents in their burrows.

Badly infected rural centers and villages may best be evacuated and the bedding and clothes of patients burned by fire.

Office International d'Hygiène Publique in 1931-2 distributed a questionnaire in regard to the value of plague inoculation. Was the scepticism felt about dead vaccine justified? Yes to judge from some of the replies. No to judge from the reply from India.

According to the Indian report a total of 147 000 vaccinations was made between 1897 and 1919 with killed vaccine and gave evidence that the risk of contracting plague had been diminished by one fourth and the risk of death to one eighth. The use of this same vaccine in Java in the epidemic of 1921-2 reduced the death rate by only one half and in some districts where the epidemic was increasing the reduction was not more than one third. In Madagascar likewise various types of killed vaccines Haffkine's aqueous vaccines and lipo vaccines have not given very encouraging results and the population depressed by the number of obvious failures have lost confidence in the efficacy of the method.

Pirie and Grasset (1938) in experimental work on rats found that none of the infected rats when tested with from 2 to 3 MLD respectively survived when killed vaccine had been used whereas all the rats survived which had been vaccinated with the living avirulent vaccine. Also the serum prepared with the killed plague organism saved none of the 6 rats tested while the serum prepared with the living avirulent organism saved 2 out of the 6.

Since the writer in 1907 demonstrated the value of avirulent cultures such cultures were not apparently employed again in human inoculation on a large scale until Otten 1934 began a careful investigation of the subject. His investigations carried on now in Java for 7 years seem to have conclusively demonstrated the great value of living avirulent cultures in the immunization of man. In 1934 Otten vaccinated 37 500 or about half the population of a district near Bandoeng Java where plague was raging. Deaths from bubonic plague as from the second week after vaccination were 23 among the vaccinated (14.5 per cent of the total deaths) and 132 among the unvaccinated (85.4 per cent of the total deaths). The vaccine was made from living cultures of the avirulent strain Tjwidej obtained from a plague rat in 1929. Later Otten stated 400 000 persons had been vaccinated with living plague without incident or accident. He believes that when plague begins to recur among the vaccinated it is a sign that revaccination is called for. He reported that among 37 435 vaccinated there were 38 deaths or 1 or per mille among 44 757 not vaccinated there were 213 deaths or 4.75 per mille and judging from the recurrence among vaccinated persons the immunity seemed to be valid for 6 to 8 months.

In a subsequent report Rosier (1938) states that 1 804 234 inoculations have been effected without any accidents. He believes that the steady decrease of the present epidemic in Java which reached its peak in 1934 is to be attributed in the first place to the use of Otten's living avirulent plague vaccine. In the regencies of Bandoeng and Soemedang out of a total population of 1 149 273 as many as 1 049 533 or 91.3 per cent were inoculated of their own free will. Subsequently Otten reported that

culture killed by heat for 15 minutes at 55°C with the subsequent addition of 0.5 per cent phenol. The dosage for an adult is usually 4 cc, given in a single inoculation.

The vaccine now supplied by the United States Army for use in endemic areas consists of 2 000 million killed plague bacilli per cubic centimeter. The initial vaccination consists of two subcutaneous injections of plague vaccine with an interval of from seven to ten days between injections. The first dose shall consist of 0.5 cc and the second dose of 1 cc of the vaccine. Additional 1 cc doses of plague vaccine may be administered whenever in the opinion of the surgeon additional stimulation of immunity is indicated.

Numerous statistics which have been published in different parts of the world would appear to have demonstrated the value of protective inoculation in bubonic plague, and the opinion is rather generally accepted today that an active immunity produced by inoculation has a distinct influence of practical importance in the prevention of the disease. The report of the Commission appointed by the Government of India to investigate the efficacy of protective inoculation against plague concluded that the evidence pointed decidedly to the value of vaccination and that inoculation sensibly diminished the incidence of plague in the inoculated population although the protection afforded was not absolute and also that inoculation diminished the death rate among the inoculated population.

Topley (1936) states the protective value of this vaccine is still difficult to assess as in most of the trials in India exact collection of statistics is notoriously difficult. The selection of the groups for vaccination has in some instances not been above criticism. Taylor 1933 has collected groups of cases in different localities and given the number of inoculated and non inoculated and the number of attacks and deaths in each group. The number inoculated amounted to 147 765 and the uninoculated 186,424. The figures suggest that the inoculations conferred protection in 4 times as many individuals as in the uninoculated. On the other hand his details show that in one locality Ahatkar Kalan 1040 were inoculated and 338 not inoculated. Nevertheless the number of cases of plague in the smaller number of non inoculated was identical with the number of cases of plague in the inoculated. The effect of the vaccination is believed in India to last for some months.

In Netherlands India Otten (1929) selected a site not yet infected but which was near an already plague infected district and it was thought that it might be attacked in the near future. However the population was vaccinated in such a manner that as nearly as possible there were equal numbers of vaccinated and unvaccinated in each house. When plague later invaded the district the returns showed that the unvaccinated were attacked in a proportion of 2 to 1 among the vaccinated.

Vogel in 1932 employed in Java Haffkine's broth vaccine. 37 224 persons received the vaccine and 39 004 did not. The mortality in the former was about one half that in the unvaccinated. However the reports of the value of the use of Haffkine's killed cultures have not always been so favorable. Thus Campbell 1938 in the study of an outbreak of plague in Africa on Lake Victoria states that mass inoculation was adopted but it did not seem to have any effect in stopping the outbreak, modifying the illness or reducing the chances of acquiring infection. Also Mitchell and Pirie in Johannesburg pointed out that from animal experiments the security engendered by the use of plague prophylactic vaccine rested on an insecure foundation and after extensive use for many years in Java it was abandoned.

Pirie and Grasset (1938) have pointed out that in some countries it has been felt that there is little advantage in using anti-plague vaccine made from killed microorganisms and that hence some concentrated their research on live vaccine. In

treated by sponging every hour or two with warm or cold water. Antipyretic drugs such as the coal tar products should in general not be employed as the heart is frequently affected early in the disease. Stimulation is frequently necessary and for this purpose digitalis, strophanthus and caffeine may be employed and seem in this disease more advantageous than alcohol. Thoulon has found digitalis of great value in treating myocarditis due to plague. In sudden collapse ammonia may be applied to the nostrils and ether injected hypodermically with favorable results. In violent or very restless cases hyoscin is frequently of service. For the headache an ice cap is preferable to drugs. Ice bags or cold applications should be applied to the buboes. Manson Bahr (1936) recommends applications of glycerin and belladonna and Choksy (1936) hot fomentations of dilute carbolic acid. The general result of experience is that energetic treatment of the buboes by caustics, mercurial inunctions or early surgical interference is painful and produces no favorable change. In Hongkong the injection into the glands of a solution of perchlorid of mercury and carbolic acid was recommended but gave only temporary benefit. Another preparation that has been advocated in India is a mixture of codeine in solution of camphor and thymol in equal parts injected subcutaneously into the bubo in doses of  $\frac{1}{4}$  to 1 cc.

When softening or suppuration occurs surgical treatment by incision and drainage may be called for but nothing is gained by too early incision. Excision of buboes is of very doubtful service and has often been followed by serious results as a rapidly fatal septicaemia. Stitt has emphasized this danger. All skin lesions and carbuncles should receive antiseptic treatment.

Morphine it is generally agreed is the best hypnotic to employ and is generally preferable to chloral and the bromides to secure sleep. Either morphine or hyoscin is sometimes necessary in the maniacal cases.

The patient should be urged to drink plenty of water in order to secure abundant elimination through the kidneys. The urine should be examined frequently and any symptoms of anuresis or acidosis treated by alkalis administered either rectally or intravenously as described in the Treatment of Cholera on p. 644.

For the vomiting cold applications to the epigastrium may be used and relief is sometimes obtained by the administration of a saline cathartic. In severe haemorrhagic cases calcium chlorid may be employed.

It is important to keep the patient prone in bed until the temperature has been normal for at least three or four days otherwise death by syncope may result. The heart's action may remain weak for a long time after convalescence and tonics and stimulants are frequently indicated. The diet should consist of broths and milk.

Tincture of iodin 5 drops every 3 hours by mouth or the application of iodin locally to the buboes or 7 minims of the tincture given in saline solution intravenously once in 24 hours has been used extensively in the Maratha plague hospital in India and its employment sometimes seemed beneficial.

during the years 1934-35 over 2 000 000 persons were vaccinated and the death rate among those vaccinated was one tenth of that among the unvaccinated

The League of Nations (1940) reports that in the 4 years 1935-39 more than 6 000 000 inoculations have been made in the Netherlands with this vaccine, and that it has considerably accelerated the decline of the disease. Otten (1941) reports that nearly 10 million vaccinations have been performed

In Madagascar, also very favorable results have been reported from the use of the living avirulent plague vaccine which was first introduced in 1933-34

The vaccine employed is known as the E V strain. In one district with a population of 10 000 of these 5300 were children under 2 years of age. Of the remainder 46 379 were vaccinated and 56 121 were not. Among the vaccinated there were 21 fatal cases 4.7 per mille among the unvaccinated 100 16.6 per mille. While the duration of immunity has not been definitely determined it is believed that 6 months is about the limit of safety and that revaccination should be performed after that time

Passa (1938) has also reported on prophylactic inoculation with this plague vaccine on the high plateau of Madagascar. In 3 years 1935-37 there were performed 600 000 711 039 and 815 453 vaccinations. The reported cases of plague were 3493 2007 and 918. A report published in 1939 by Vogel and Riou shows that in Madagascar prophylactic vaccination has been on a large scale during 1936 and 1937. The living E V vaccine was used and the total of vaccinations was 815 453 for a population of 1 031 393 inhabitants or 77.5 per cent. The table of monthly graphs for the years 1933-38 shows a marked decrease of cases for 1936-37 by comparison with the years 1933-35 inclusive. The results are claimed as showing the effect of the new method of vaccination on a large scale

Anchezar (1938) shows that the E V strain of Girard which was originally isolated from a case of bubonic plague corresponds to *B. pestis* in all respects. It is avirulent for laboratory animals and inoculation of large doses kills animals by toxæmia

Girard and Robic (1938) also report that a huge program of plague prophylaxis in Madagascar by means of this living avirulent E V vaccine has been carried out during the past 3 years. Over 2 000 000 vaccinations have been performed. The killed vaccine which was originally used had given mediocre results. With the living vaccine on the other hand an 80 per cent reduction of mortality resulted

Otten (1938) points out that only certain avirulent strains are valuable for human protective inoculation and that the antigen potency must be watched and preserved. He also states that it is obvious that certain of the avirulent strains that have been studied at the Haffkine Institute have evidently been of inferior immunizing power

Grasset (1942) has also reported upon the use of the live vaccine in South Africa. The vaccine suspension consisting of a 24 hour growth at 37°C was adjusted to a concentration of 1 000 million organisms per c.c. and made from the two avirulent strains carefully tested for purity E V and Tywidej strains

Schutze (1939) thinks that the protective power of the smooth avirulent living vaccine depends upon its capacity for prolonged survival in the inoculated animal. With killed cultures he thought he could show that the rough strain is more quickly suppressed and has therefore less time to elaborate antigen. However Otten (1938) has shown that the degree of virulence whatever relation to antigenic structure may exist cannot be accounted for by the morphology of the colony

#### TREATMENT

**Symptomatic Treatment.**—The treatment of plague is largely symptomatic. The patient should be kept in bed given good nursing and fresh air. An initial purgative is generally advisable. The fever should be

Drug	Number of cases treated	Number of deaths	Case mortality
Sulphathiazole	147	33	22.4
Sulphapyridine	70	21	30.0
Controls—treated with usual hospital treatment of iodine intravenously	140	80	53.6

## CASES WITH PLAGUE SEPTICAEMIA AT THE COMMENCEMENT OF TREATMENT

Drug	Number of cases treated	Number of deaths	Case mortality
Sulphathiazole	62	6	4.9
Sulphapyridine	33	19	57.5
Controls—no treatment	75	68	90.8
Serum treatment			60.0

Plum (194) in Nairobi has employed sulphapyridine for treatment and reports that if it was given sufficiently early and in large doses it acted almost specifically. The available dose used in an adult case was 4 tablets on admission and thereafter two tablets every 2 hours until the temperature had been normal for 24 hours. In this case treatment on the first day of the disease the mortality was about 100% on the second day of the disease 20% on the third day 3% and on the fourth day and 0% on the fifth day.

**Treatment**—The following suggestions for treatment have been made to the Medical Corps of the United States Army.

**General**—Morphine as indicated for restlessness and delirium. Force fluids by mouth or parenterally for toxemia.

**Chills**—It is most important to initiate the treatment without delay after the diagnosis has been established. In order to prevent the development of a continuous septicaemia the therapy must limit the blood levels of from 5 to 10 mgms per cent during the first four or five days of the disease. Sulfadiazine is the drug of choice. Sulfathiazole is less effective but may be used when sulfadiazine is not available. To achieve the desired level the drug should be given as follows:

**By mouth** Initial dose 4.0 g m (6 grs); t.p.i. 2.0 g m (3 grs) four hours and night subsequent doses 5 to 2 gram (2 1/2 to 30 grains) every four hours day and night until temperature is normal. Then continue 2.0 g m every four hours for at least 5 days after the temperature is normal. A gradual reduction in the blood level of from 10 to 5 mgm per cent is indicated when the patient shows improvement. During treatment must be determined individually. The fluid intake must be regulated to insure a daily urinary output of at least 1500 cc.

**By intravenous route** In fulminating cases where treatment has been delayed sodium sulfadiazine (5% solution in distilled water) should be tried as follows: Initial dose 0.5 g m (5 grs) per kilo of body weight given slowly subsequent doses 0.05 to 0.1 gram per kilo every six hours. Changing to oral dosage as soon as possible.

Wayson and McMahon (1944) in an important study have shown that 15 guinea pigs that were clinically dead finally with ulcerated plaques and developed plague necropsy were killed 21 days after inoculation. One died with ulcerated plaques in the kidney. Thirteen untreated controls died of plague after similar inoculation. Eleven guinea pigs infected with plague by bites developed the disease but recovered and were treated with sulfadiazine and showed no evidence of the infection at necropsy 21 days after inoculation. Nine untreated controls which were infected and finally never developed the disease and died. They point out that the drug

Intravenous injections of a number of antiseptics have been advocated among these have been mercurochrome, quinone and catechin. Others have been iodine, neosalvarsan, Bayer 205, electragol, eusol, gonacrine, methylene blue and formalin. Only for a time were favorable results reported from intravenous injections of mercurochrome. A 1 per cent solution in water was given in doses of 2-3 mg. per kilo of body weight. The dose especially recommended was 20 cc. of a 1 per cent solution. In Java, a substance known as ommadin in 2 cc. doses has been employed. Cairns and Naidu (1937) have shown that mercurochrome has no favorable influence on plague in rats or rabbits and it has been rather generally accepted that the drug is not efficient in the treatment of human cases.

*Bacteriophage* has been tried in the treatment of plague by D. Herelle and Naidu and their associates. In bubonic cases it was recommended that 2-3 cc. should be injected subcutaneously into the bubo on the first and again on the second day. In septicaemic plague 3 cc. or more have been given intravenously. Naidu and Avari (1937) in India employed a bacteriophage which lysed a 24 hour plague culture in less than 2 hours but it proved quite ineffective in practice. Guilliny has treated cases of pneumonic plague in Madagascar intravenously with a test phage but patients so treated did not seem to receive any benefit. There have been a few more favorable reports of its use. Advier (1933) reported treating 35 cases of bubonic plague with a phage obtained from a patient in Senegal. Twenty of them recovered. Couvy and his associates also report treating 21 severe cases of bubonic plague with 15 recoveries. Sorrel (1937) has used it by injecting it either intravenously or subcutaneously directly into the bubo; the results did not appear to differ from those obtained by serum treatment. Robic (1937) on the other hand found the phage favorably reported upon in Dakar, as useless in Madagascar in treatment of either the bubonic or pneumonic forms.

*The Sulfonamides*—On account of the value of some of the sulfonamide compounds in streptococcal infections several of these have recently been tested in plague. Sokhey and his associates (1939) have found that prontosil and M & B 693 showed little or no curative power in experimental infection in rodents. However, Durand (1939) reports that dagnan or M & B 693 (para aminophenyl sulphamido-pyridine) may be fed to mice daily and that the drug was fairly well tolerated and that such mice when later inoculated with plague survived. Schutze (1939) and Girard and Girard (1939) also reported good results in the treatment of rodents with this preparation. Three cases of plague treated with prontosil by Vine (1939) were all said to have recovered. Wagle, Sokhey and Dukshit have recently employed the sulfonamides in the treatment of Bubonic and Septicaemic plague. Their results may be recorded as follows:

valescent cases and also those in whom the illness had already lasted for 6 days or more

The observations were thus restricted to the most acute cases within the first 5 days of the illness. Every alternate case was then treated with serum. Four hundred cases under his observation were treated in this way. In the serum cases the mortality was 63.5 per cent and in the 200 controls the mortality was 74 per cent. There was thus a difference of 10.5 per cent in favor of the serum cases. In a previous series of 238 cases treated with the serum the mortality rate was 59.2 per cent.

By comparing the time of death after admission between the serum and the control cases it was found that whereas 79 per cent of all death among controls occurred within 4 days after admission the proportion was 58.2 per cent among the serum cases—a difference of nearly 21 per cent the serum having considerably prolonged life. Of 243 cases treated in private practice with the serum the mortality was as low as 40.7 per cent.

Out of the entire 1081 patients subjected to the serum treatment 537 died and 544 recovered the mortality rate being 49.6 per cent. 613 of the cases were treated in hospitals in which the case mortality was 57 per cent and 468 were private cases in which the mortality rate was 39.9 per cent. A very striking feature is the difference in the mortality rate according to the stage of the disease at which the serum was injected. Of 516 patients treated on the first day 220 recovered the mortality being 30.3 per cent. On the second day of illness 300 cases were treated 142 recovering or a mortality of 52.6 per cent. The table on p. 708 also shows the increased mortality in the cases treated later than the second day of the disease.

The general mortality of plague at that time in India were estimated at 89.9 per cent. He concluded his observations by stating that the success of the treatment lies in applying the serum very early. Among patients subjected to the treatment within the first few or even 24 hours it is noticed that the whole course of the disease becomes altered. The normal duration of the disease from about 8 to 10 days is reduced to 4 or 5 days. Serious complications of the nervous, circulatory and other systems are averted. The buboes become absorbed and convalescence is more rapid. After 48 hours the serum does not appear to influence the course of the disease perceptibly.

INCREASED MORTALITY IN CASES TREATED AFTER SECOND DAY OF DISEASE

Duration of ill	Number	Recovered	Case mortality per cent
First day	316	220	30.3
Second day	300	142	52.6
Third day	246	9	63.0
Fourth day	105	45	5
Fifth day	52	20	61.5
Sixth day	4	6	57.1
Seventh day	4		0.0



treatment should begin as soon as the fever and buboes have developed and should continue through the febrile period. A blood level of 4-7 mg per cent of the drug was usually maintained but no attempt was made to determine the level required for therapeutic efficiency.

**Surgical**—Hot wet applications to the bubo may hasten localization of the infection. Incision should be delayed and in any case not performed until localization is complete in order to avoid blood stream infection.

**Serum Treatment**—In order to understand the action of plague immune serum in the treatment of plague it is necessary to understand the action that such an immune serum has upon *Past pestis*. The writer demonstrated in 1907 that the plague immune serum prepared with living virulent organisms is neither antitoxic nor bactericidal in its action against the plague bacillus *in vitro* but that it has the power of preventing the development of the plague bacillus and of preventing its multiplying and may be termed anti-infectious in its action. With this knowledge it is not difficult to interpret the results which are obtained in the serum treatment of animals experimentally infected with plague and we find that the success of the serum treatment appears to depend particularly upon the number of plague bacilli in the animal organism at the time of the inoculation of the serum; that is upon the length of time the serum is injected after the infection has occurred. If the organism is already overwhelmed with bacteria at the time of the introduction of the serum almost no favorable change will be noted in the course of the disease because the serum is merely anti-infectious and is not antitoxic.

Thus of a series of rats inoculated by the writer with immune serum at the time of their infection with plague bacilli 60 per cent survived and 40 per cent succumbed to the infection while of another series which were inoculated with the serum 24 hours after the plague infection only 40 per cent survived and 60 per cent died. In another series of experiments in which larger doses of serum were employed and a less severe method of infection the animals were inoculated with the serum in 3 series: one at the time of the infection, a second 4 hours following the infection and a third 48 hours after the infection. The mortality in the first series was 10 per cent, in the second 40 per cent and in the third 66.6 per cent. Similar results have been obtained with monkeys and sometimes it is possible to save those animals which have previously been infected with plague by the inoculation of plague immune serum injected as late as from 12 to 24 hours after the time of the infection provided large doses of the serum are used. With rats it has been shown that if large doses of the serum are used even animals in which the disease is fairly well advanced may sometimes be saved by the serum.

**Result of Treatment in Man**—Turning our attention to the treatment of human cases of plague with serum we find somewhat similar results reported. Choksy in India who has had a very extensive experience with the serum treatment of plague states that much depends upon the early and free use of the serum. In patients treated on the first day or within a few hours of the onset of the symptoms one injection of 100 cc followed by another after 6 to 8 hours and then if necessary by a third after a similar interval would cut short the attack if the case were not pneumonic malignant or septicaemic. He also emphasizes the fact that the earlier the serum is used the more efficacious it is and that if good results are to be obtained from serum therapy, the patient must be treated on the first day of the illness. He admits that the serum cannot favorably influence all types of plague or even the malignant forms of the bubonic type but he shows that it is the only treatment capable of saving a large proportion in a certain class of patient.

In a more recent publication he summarized his observations regarding 1081 cases. There were eliminated from the observations septicaemic, pneumonic and moribund cases as well as convalescent and semicon-

point out that none of the sera which had been previously employed produced a case mortality below 60 per cent. Such work is exceedingly encouraging.

It should be borne in mind that we have experimental data in animals showing that a proper immune serum *will* protect a certain percentage of the animals if given early in the stage of the infection.

*Prophylaxis and Pneumonic Plague*—In an epidemic of pneumonic plague the public health campaign must center upon the early detection and isolation of cases and of conducting evacuation of infected areas and masking. Every case of primary pneumonic plague constitutes a very dangerous focus of infection. The fully virulent microorganisms are present in enormous numbers in the sputum, often in almost pure culture, and the plague bacilli are also expelled in large numbers into the surrounding atmosphere by coughing. As a result any person entering a ward containing cases of plague pneumonia is liable to contract the pneumonic form of plague.

Plague bacilli are not killed by freezing for long periods of time, and hence epidemics of pneumonic plague are particularly serious during cold weather. In order to prevent the spread of pneumonic plague the cases must be recognized early and rigidly isolated. Suspected cases should also be isolated. There must be separate hospitals for plague patients for suspected cases and for contacts. Sanitary cordons should be established against infected areas and there should be strict medical inspection and quarantine for five days. Buildings such as schools, churches, theatres, factories and markets should be closed. The pneumonic plague hospital must be built or arranged so as to admit of individual isolation. No patient should be transferred from the suspect hospital to the plague hospital until a positive diagnosis of plague has been made. The pneumonic plague hospital for suspected cases must also admit of individual isolation of patients.

Houses in which pneumonic plague cases occur should be thoroughly disinfected in the manner described for bubonic plague. The excretions and particularly the sputum must be thoroughly and carefully sterilized. All soiled linen must be disinfected, also and walls and floors should be mopped with 1 : 1000 bichloride solution.

It has been advised that the sanitary staff be inoculated with plague vaccine. However they should not rely upon such protective inoculation. Teague and the writer found in extensive experiments with monkeys that only about 10 per cent of the vaccinated animals were protected against plague infection by inhalation. The remaining 90 per cent of the animals died of pneumonic plague. Wasilewski in the epidemic of pneumonic plague in eastern Siberia in 1921 also concluded that antiplague vaccination has no favorable influence in pulmonary plague. For the passive immunization in a household of individuals that have been exposed to infection the injection of 50 cc of plague immune serum may be employed. Doctors, nurses and attendants should be provided with face masks made of 8 layers of gauze or 4 of cheesecloth which should always be worn when at work in the vicinity of pneumonic plague cases. Goggles should be worn in examining cases also and gloves when autopsies are performed. A cotton gown should be worn in the wards and removed on leaving them. Attendants are advised not to shave immediately before entering the wards to attend patients on account of the danger of infection through the slight abrasions on the face. Individual masking

Simpson, in his *Treatise on Plague*,<sup>1</sup> summarizes his remarks in regard to treatment with the statement that if the serum is injected intravenously and early it appears to give the patient a better chance of recovery than any pharmacopoeial drug and in some instances the state of the patient after the injection is so much improved that it can be attributed only to the action of the serum.

In 1913 the British Plague Commission in India came to the conclusion that it appeared that the administration of the available sera was not a practicable means of bringing about any material diminution in the mortality of plague in India but nevertheless the results of their analysis seemed to show as the others discussed have that if the serum can be given early enough in the disease and if the infection is not too severe a beneficial effect may be often obtained. The results of serum treatment in plague however are frequently uncertain and it must be borne in mind that it is only within a narrow limit of time that its use in man as in animals is efficacious.

Plague immune serum is still used to some extent for treating bubonic plague in India but the reports are by no means unanimous as to its value. The dose recommended and given has frequently been from 30 to 40 cc no matter how serious the condition of the patient. Nevertheless Dawson has reported that of 50 patients in apparently moribund condition 16 recovered after receiving the serum. Apparently in many instances no attempt has been made to demonstrate the immunizing power of the serum employed by experiments upon rats. *Satisfactory plague immune sera cannot be prepared in horses unless satisfactorily virulent living cultures are inoculated. Satisfactory immune sera cannot be prepared with killed cultures of the plague bacillus or with extracts of the bacillus.* Although this fact had been emphasized in earlier years by the writer some laboratories on account of the difficulty of inoculating the partially immune horse with living virulent cultures have manufactured their serum only with killed cultures. Thus Pirie and Grasset (1938) pointed out that the plague immune serum at the South African Institute was formerly prepared only with killed cultures. They have since demonstrated the great value of serum prepared by the inoculation of horses with living avirulent cultures. This is now the standard method of the Institute.

Sokhey (1938) has recently prepared at the Haffkine Institute a new anti plague serum. It was tested from the middle of January to the end of April 1938 during an outbreak of 124 cases. Every alternate admission to the hospital was treated with serum.

Others were treated with intravenous injections of iodine solution. No selection of any type was made but during the third week of the trial all patients admitted were treated with serum. Of the 69 patients treated with serum 19 died of plague giving a percentage mortality of 27 per cent. Of the 55 cases treated with iodine injections 36 died giving a percentage mortality of 65 per cent. In a previous trial conducted at Hyderabad with the same serum 94 patients were treated with the serum with 24 deaths and a percentage mortality of 25.5 while among 80 controls there were 50 deaths giving a percentage mortality of 62.2 per cent.

Clinically the administration of the serum produced very striking results. The general condition of the patients improved very rapidly with the disappearance of toxic symptoms. It is stated that the results show that there has been obtained a serum of a high curative value. They

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in this disease is perhaps more effective than in protection against influenza on account of the great difference in size of the etiological factors involved. In influenza, the virus, being ultra microscopic would probably not be interrupted to the same extent by the mask as the plague bacillus.

**Spread of Pneumonic Plague and Plague Pneumonia**—The danger of the spread of pneumonic plague and its high fatality is emphasized by the following figures

Manchuria 1910 60 000 cases all fatal	Ecuador 1939 16 cases 15 fatal
Manchuria 1920-1921 10 000 fatal cases	South Africa (Kalashi) 1941 37 cases
California Oakland 1919 13 fatal cases	36 fatal
Los Angeles 1924 32 cases 30 fatal	Nairobi 1942 131 cases 19 fatal
Madagascar 442 cases all fatal	

It has been noted that when secondary pneumonia develops in the course of bubonic plague in India the Philippines, or other hot countries it is not followed by primary plague pneumonia outbreaks. This is thought to be due to the fact that the windows are wide open and the relative humidity low conditions which are the opposite of those which existed in Manchuria where the intense cold made the closing of windows necessary and where the air of rooms or wards was saturated with the moisture from the occupants. As the main consideration for the spread of pneumonic plague seems to be high relative humidity, it would seem advisable that hospital wards should be constructed so that the air supplied by artificial ventilation would be very dry.

In the spread of respiratory diseases the recent investigations of Wells and Stone (1934) upon air borne infections are of interest in which they point out the importance of dried infected droplet nuclei derived from droplets less than one tenth of a millimeter in diameter. Wells (1940) has emphasized the destruction of droplet nuclei infection by ultra violet light as a promising means of preventing droplet infections in hospital wards.

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Vail (1914) reported conjunctivitis tularensis and from his patient Wherry and Lamb by animal inoculation made the first isolation of *Past tularensis* from man. The two latter (1914) also reported the first isolation from wild rabbits.

Francis (1919) identified the deer fly fever of Utah with the plague like disease of rodents of California and gave in 1921 the name of tularaemia to the disease on account of the bacteriaemia. He isolated *Past tularensis* from fly bitten human cases, wild jack rabbit and one ground squirrel demonstrated agglutinins in the serum of human cases and transmitted infections in laboratory animals with *C. discalis*. His work and the stimulus of his leadership have been predominantly responsible for further investigations and dissemination of knowledge concerning the disease.

Parker and Spencer (1924) established *Dermacentor andersoni* as a host and vector for the disease in man and rodents, demonstrated (1926) hereditary transmission in *D. andersoni* and showed the rabbit tick (*Haemaphysalis leporis palustris*) to be a vector for rodents.

Ohara (1925) reported a febrile disease of man in Japan associated with a disease of wild rabbits and Francis and Moore (1926) showed it to be tularaemia.

**Geographical Distribution.**—By 1928 650 cases had been reported in the United States being contributed by the District of Columbia and all states except Wisconsin, Washington and some of New England. Up to 1942 cases had occurred in all states but Vermont. In Japan the disease was recognized in 1905. In Russia (1928) it was noted in native hunters of the water rat *Arvicola amphibius* which are skinned for their fur. Russian observers have noted that the infection is widespread among rodents in places where no examples of the human disease have been discovered. The League of Nations reported its occurrence in Norway in 1929 and Olin and Sehlstedt (1931) have encountered 31 cases in Sweden. The infection has also been observed in Canada 1930 and in Austria 1935, Germany, Czechoslovakia, Moravia and Turkey 1936. Francis (1937) notes that human cases have been found in 46 states and in Washington, D. C. in the United States. Groups of cases have sometimes been noted in different localities. Thus within a few months in 1928 31 cases of tularaemia were reported in one county in Tennessee and 53 in the city of Dayton, Ohio. The increased frequency of reported cases has suggested an even greater prevalence. Foshay (1940) points out that in the fall of 1936 there occurred a large endemic outbreak in the Cincinnati region with about 140 cases in the 6 week period between November 15 and December 31. Similar increases over the usual normal number were noted the same year as far north as Dayton, Ohio and in southeastern Indiana and northern Kentucky.

#### ETIOLOGY AND EPIDEMIOLOGY

**Etology.**—*Pasteurella tularensis* (*Bacterium tularense*) is a small (0.3 to 0.7  $\mu$  long) non motile, Gram negative, non sporebearing, pleomorphic (bacillary, coccoidal and bipolar) organism and gives the appearance in

## Chapter XIX

# TULARAEMIA

### DEFINITION AND SYNONYMS

**Synonyms** —Plague like disease of rodents deer fly fever conjunctivitis tularensis rabbit fever glandular type of tick fever, Ohara's disease (Japan)

**Definition** —A primarily fatal bacteriaemic plague like disease of various rodents especially rabbits and hares caused by *Pasteurella tularensis* (*Bacterium tularense*) It is highly infectious and is transmitted to man from rodents by the bite of a fly or tick or by contamination of the skin or conjunctiva with tissues or body fluids of infected rodents flies or ticks

The site of infection in man is usually marked by a necrotic punched out ulcer which is associated with a regional lymphadenitis that tends to chronicity The onset of the constitutional symptoms is sudden with rigors the fever is irregular, lasts two or three weeks and shows an early temporary intermission convalescence is prolonged

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** —The disease passed unrecognized as an entity for years being considered, probably most frequently, as influenza sepsis typhoid fever, pneumonia meningitis tuberculosis or sporotrichosis Available records enable us to identify it in the United States only since about 1907 and in Japan, for about the same period although in the latter country Homma (1837) described an intoxication caused by rabbit meat

Martin (1907) in a personal communication described cases in Arizona that are now recognized to have been human oculo glandular and ulcero glandular tularaemia acquired by dressing jack rabbits and one of his cases showed anti tularense agglutinins in 1925

Pearse (1911) differentiated clinically the deer fly fever of Utah incriminating *Chrysops discalis* as the transmitter

McCoy (1911) described a plague like disease of rodents in ground squirrels (*Citellus beecheyi*) from Tulare County California

McCoy and Chapin (1912) discovered the causative organism in ground squirrels named it *Bacterium tularense* cultured it transmitted the infection to various rodents by feeding nasal inoculation and injection of blood, and reported positive complement fixation and agglutination tests for human sera The name is from tule (Aztec) a variety of large bulrush found in Tulare County in extensive marshy beds which caused the Spaniards to call the region Tulares

*p l i s* (the rabbit tick) as previously mentioned and probably also by lice Francis and Lake having effected transmission in the laboratory with *Haemodipsus ventricosus* (the rabbit louse) in 1921 and with *Polyplax serratus* (the mouse louse) in 1922. *Ceratophyllus acutus* (the squirrel flea) was found by McCoy (1923) to be but a feeble vector. There is also the possibility of infections through injuries during fights or from attacks of infected carnivores. Francis and Lake (1922) showed *Cimex lectularius* (the bedbug) to be an efficient vector among mice in the laboratory and Wavson (1914) called attention to the possibility of *Stomoxys calcitrans* (the stable fly) and *Musca domestica* (the house fly) being mechanical vectors.

Although the usual method of infection is probably inoculative it is said that *Past. tularensis* may sometimes penetrate the unbroken skin. Laboratory animals may be infected by rubbing pathological material from other animals on the shaven skin and the disease then pursues the usual

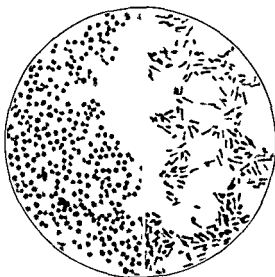


FIG. 71.—*Past. tularensis*. Noting from ocular film of mouse (Photomicrograph by M. J. G. R. Callahan, M. C. U. S. A. by courtesy of Surgeon General U. S. P. H. S.).

course. White mice can acquire the disease by eating the liver of infected rabbits or by eating infected bedbugs and the faeces of such bedbugs is infectious. Guinea pigs can be infected by the subcutaneous injection of the urine of infected white mice.

*C. disalis* is found especially in Utah and the vicinity and infections by it are probably mechanical as it loses its power of transmission after about five days.

*D. andersoni*, a native of western North America serves not only as a vector but is also an important reservoir since it remains infective throughout its life and *Past. tularensis* is widely distributed in its body being found in the lumen of the gut in the cells of the gut wall in the circulating body fluid and in the faeces. It harbors the disease through the winter and the infection is transmitted to the eggs. Other species of



stained preparations from tissues of being surrounded by capsular material. It stains well with ammonium oxalate crystal violet in films from cultures and tissues but Giemsa's stain is advisable for tissues. In cultures, short bacillary forms up to  $2\mu$  in length may occur, or coccoid forms may later predominate. On account of the small size of some of the organisms they will pass through some of the coarser bacterial filters.

It is an obligate aerobe and will not grow on ordinary laboratory media. Cystine is necessary for culture and suitable media are blood glucose cystine agar and coagulated egg yolk. Growth in slow small colonies appear about the third day and are smooth rounded and translucent. Optimum for culture are a temperature of  $37^{\circ}\text{C}$  and a pH of 6.8 to 7.3. Fermentation of glucose levulose mannose, maltose and glycerol occurs with formation of acid but not of gas.

It is killed by  $56\text{--}58^{\circ}\text{C}$  in cultures and, in ten minutes in spleen tissue. One per cent tricresol kills it in two minutes when rubbed into infected spleen tissue. Cultures are rendered nonvirulent in twenty four hours when mixed with 0.1 per cent of a 37 per cent solution of formaldehyde in physiological saline. Francis has kept the organism alive and virulent in glycerinated guinea pig spleen tissue at  $-14^{\circ}\text{C}$  for 10 years. It has been found to remain virulent in the faeces of infected bed bugs for 75 days. No toxins have been demonstrated.

Cross agglutination occurs in connection with the antisera for *Past. tularensis*, *Brucella melitensis* and *Brucella abortus* but may fail with sera of high titre as well as with those of low titre. This matter is considered further under undulant fever. About 23 per cent of tularaemia sera were found to agglutinate *B. melitensis* and *B. abortus* to some degree and in some instances to the same dilution that they agglutinated *Past. tularensis* but usually the anti tularensis titer is much higher than for the others. The cross agglutination also usually developing more slowly. About 33 per cent of undulant fever sera agglutinated *Past. tularensis* to some degree.

**Epidemiology**—Natural infections occur in wild rodents the most important reservoirs being the cottontail rabbit (*Sylvilagus* sp.) the jack rabbit (*Lepus* sp.) and the snowshoe rabbit (*L. bairdi*). Domesticated rabbits have not been found naturally infected. Other reservoirs are the California ground squirrel (*Citellus beecheyi*) as reported originally by McCoy and Chapin, wild rats reported (1925) from Los Angeles California by Dieter and Rhodes and meadow mice (*Microtus californicus aestuarinus*) reported (1927) from Contra Costa County California by Perry. The opossum *Didelphis* has also been found naturally infected.

*H. Cinnabarina*, the bird tick, has been found infected during epizootics in game birds in the western United States. (Philip 1935.)

Jellison, Kobbs, Butler and Weaver (1942) have reported upon epizootic tularaemia in the beaver *Castor canadensis*; the diseased animals being found in Montana streams. *P. tularensis* was recovered from the tissues of the dead beavers. Water from four streams was shown to be contaminated with *P. tularensis* and in one stream which was contaminated the infection persisted for at least 33 days after any beavers were known to be present. Their data suggests two new questions concerning the epidemiology on tularaemia: a) The possibility that under favorable conditions epizootics may occur in local animal populations without the aid of blood sucking parasites. b) The possibility that stream water contaminated with *P. tularensis* may not only be a source of infection for beavers but occasionally of human infection.

The disease is transmitted and maintained in animals by *Dermacentor andersoni* (the wood tick), *Chrysops discalis* (the horse fly or deer fly), *Haemaphysalis leporis*

Wild rabbits—shot and dressed 247 cases bought or sold in markets 220 cases skinned dressed or cut up 1078 cases Fly bite (*Chrysops d. scalaris*)—68 cases Tick bite (*Dermacentor a. d. rsoni*)—42 cases in Montana and surrounding states Tick bite (*Dermacentor ta. labilis*)—73 cases principally in southern states Laboratory animals autopsied—39 cases Sheep contact and thereby contact with wood ticks and their faeces in shearing herding and butchering in the Northwest—2 cases Tree squirrel—skinning 10 bite clawing 1 Cats—bites 5 scratching 2 Coyotes—bite 1 skinning 1 Opossums—bite 1 skinning and dressing 9 Dogs—bite 1 picking ticks from dog 1 Skunks—bite skinning 2 Ground squirrels—bite in Montana 1 Hogs—bite 1 autopsy 1 puncture by splintered bone 1 Ground hogs—skinning 2 Muskrat—skinning 2 Quails—dressing 2 Sage hen—dressing 1 Fox—skinning a red fox and several small rodents 1 case in Maine Deer—skinning and dressing 1 case in New York Bull snake—skinning 1 Water rats of Europe (*Arct. alba amphibius*)—skinned for their pelts in Russia about 1000 cases

Foshay (1940) in an analysis of 600 cases found the source of infection to be from rabbits in 519 cases and from squirrels in 10 cases and in the remainder the source of infection was variable

Infection has also occurred in laboratories especially in those who have performed autopsies on infected animals and the greatest care in technique is necessary to avoid them Ingestion of insufficiently cooked wild rabbit meat is said to have caused 6 cases of which 12 died

Aside from laboratory infections the appearance of cases shows a certain seasonal incidence dependent upon the period of greatest activity of *D. andersoni* (March to August) and *C. discalis* (June to September) and the open season for hunting rabbits

Infection from man to man has not been reported either by contact operative procedures or insects The only record of such a possible transfer is that of Harris (1926) in which a mother is believed to have contracted tularaemia through a prick of her thumb received while dressing the ulcer on her fly bitten son the incubation period having been about 24 hours

The disease is found especially in rural districts among those exposed to ticks and flies and among those handling wild rabbits such as hunters marketmen housewives and cooks

About 75 per cent of cases occur in males and the extreme age limits reported have been and 73 years

### PATHOLOGY

**In Animals**—There is frequently a rapidly fatal septicaemia There may be glandular enlargement with focal and diffuse acute necroses in the spleen liver lymph nodes bone marrow and lungs in infected guinea pigs rabbits and white mice but the animals may die before such changes as are found in the late stages in man can develop *Past. tularensis* invades the fixed tissue cells having been demonstrated in the hepatic cells of the guinea pig and mouse and in the cells of the intestinal epithelium of the tick and bed bug

Bacteraemia is particularly prominent in laboratory animals the lethal dose of heart blood being as small as 0.000.000 or cc The coelomic fluid of infected ticks and bed bugs is usually rich in organisms

ticks *D. variabilis*, *D. occidentalis*, *Ixodes Ricinus* var *californicus*, are said also to be able to act as vectors. Olin (1937) who has studied a serious outbreak in Sweden in which 137 cases occurred among the peasants who went bare footed in summer believes the infection was transmitted by mosquitoes. He reports that 4 species of *Aedes* and one of *Theobaldia* would transmit the infection experimentally to guinea pigs.

High susceptibility occurs in man, monkey, rabbit, ground squirrel, guinea pig, mouse, woodchuck, opossum, grouse and young coyote, slight susceptibility in the rat, cat, sheep, and goat. The horse, cow, hog, dog, fox, pigeon and chicken are not susceptible.

The disease in man depends on the presence of the infection in wild rodents, especially rabbits, and is often known to be associated with the discovery of sick and dead rabbits in the vicinity. The organism may

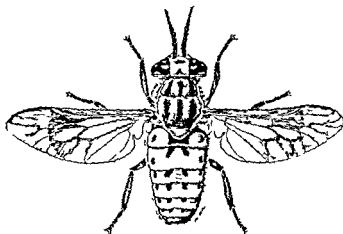


FIG. 172.—*Chrysops discalis*. A transmitting agent of tularemia.

enter through an abrasion of the skin or through the conjunctivae or by the bites of insects or animals. Scratches, abrasions, thorn punctures, etc., have been reported at the site of infection.

Infection is acquired especially from infected tissues or body fluids and may occur in connection with dressing or handling rabbits (which accounts for about 75 per cent of reported cases), also of woodchucks, or laboratory animals, or with the crushing or handling of ticks or flies. Reported infections from bites of insects comprise those of the horsefly *C. discalis*, or of the wood tick *D. andersoni* and *D. variabilis*, which have become infective from feeding on diseased rodents. In ticks, the organisms may be harbored for long periods within the body cells and coelomic fluid as well as in the lumen of the gut. Hereditary transmission has been demonstrated from infected ticks to their eggs, larvae and nymphs.

Francis, (1937) in 1824 cases reported in the United States gives the source of the human infection as follows:

varying amount. The adjacent inguinal lymphatic glands are swollen and frequently there are haemorrhages in the region of the glands. The glands are often softened and necrotic and upon section the center is yellow and caseous in appearance. The spleen is greatly enlarged and congested and both it and the liver which is also congested contain very numerous necrotic foci which vary from pin point to about 1 mm. in diameter. The lungs and also the kidneys frequently show haemorrhages. Very large numbers of a very minute round or rod shaped organism are found in microscopic preparations from the heart's blood and the liver, spleen and swollen lymphatic glands. These organisms stain with carbolfuchsin and anilin gentian violet and with Giemsa's solution. Prolonged staining gives the best results. They are decolorized by Gram's stain and are apparently not motile. In stained preparations the organisms

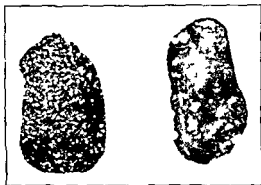


FIG. 174.—Gross section of spleen showing a splenic infarct (left) and subcapsular infarct (right) in tularaemia (Fuchs) (Army Medical Museum No. 4035).

are found lying inside well defined round or oval clear unstained areas sometimes several or a dozen or more organisms are found in such a clear space. In size the rod shaped organisms in the body vary from about 0.3 to 0.7  $\mu$  in length and 0.2 to 0.3  $\mu$  in width. The round forms vary from 0.2  $\mu$  to 0.5  $\mu$  in diameter. The sections show necrosis and polymorphonuclear infiltration in the vicinity of the site of the inoculation followed by an extensive proliferation of the vascular endothelium.

In the lymph nodes liver and spleen there are milium foci formed by accumulation of mononuclear cells followed by necrosis and infiltration with polynuclears. In the liver there are in addition foci of necrosis without cell accumulations or capillary lesions. These changes are constant and there may be lesions of much the same character in the adrenal the heart the lungs and the testicle. After death *B. tularensis* is present in great numbers. There is a general infection of the blood vessel endothelium and the organisms may be found in vessels of any part of the body. In addition the organisms pass from the endothelium into the cells of the liver which they gradually destroy and replace forming

The gross lesions in guinea pigs after abdominal inoculation strongly resemble those of plague. In fact, the writer (1921) showed in a series of successive inoculations carried out during several years with several hundred guinea pigs that it was frequently impossible to tell at autopsy from the macroscopic lesions in many instances whether *Past tularensis* or *Past pestis* was the infecting organism. The crucial test for diagnosis of any plague material has been regarded as the power of the plague bacillus



FIG. 173.—Guinea pig infection with *P. tularensis*.

to infect a rat or guinea pig when the material is rubbed into the shaven skin of the animal. However this test of power to infect especially in a guinea pig would not differentiate tularensis infection from plague. When a guinea pig is inoculated by scarifying the skin of the abdomen with a scalpel and rubbing the scarified area with a small portion of the spleen of a tularensis infected animal death usually occurs on the third or fourth day after the inoculation but the day of death may vary between 3 to 6 days after inoculation.

At the autopsy there is induration and thickening about the point of inoculation subcutaneous congestion oedema and often haemorrhages of

fragmentation polymorphonuclear infiltration and a base infiltrated with small lymphocytes. The regional lymph glands show focal and diffuse necroses with leucocytes debris and nuclear fragments. In the spleen bordered by normal pulp there are superficial and deep necrotic foci containing amorphous material nuclear fragments and a few leucocytes. Absence of Langerhans cells was noted by both Goodpasture and Simpson. The liver may show foci of necrosis of the hepatic cells at first the area is filled with large mononuclears when more advanced with many polymorphonuclears and nuclear fragments. The lungs may present small necrotic foci or white plaques on the pleura focal necroses may be present or there may be bronchopneumonia of any degree even to the involvement of almost an entire lobe the alveolar walls are infiltrated with oedematous exudate and large mononuclears and the alveolar contents consist of a few leucocytes and red blood cells and a small amount of fibrin.

In the subacute stage the lesions may become granulomatous in type and bear a strong resemblance to tuberculosis. There is central necrosis surrounded by a layer of radially arranged epithelioid cells and fibroblasts and a peripheral zone of lymphocytes with a few giant cells. This applies to the primary ulcer the lymph glands (both regional and deep) the subcutaneous nodules spleen liver lung and adrenals.

Talat Vassfi (1940) has reported that he isolated strains of tularaemia termed Hamza bey and Ceylon strains from a brook during the study of a tularaemia epidemic in Thrace in 1937 and that he obtained a powerful endotoxin from cultures of these strains. The potency of the toxin was shown from the reported experiments in mice guinea pigs and rabbits but the lesions in guinea pigs either from the injection of the toxin or of the organisms themselves were not described. Obviously great care should be taken in the identification of organisms isolated from water.

### SYMPTOMATOLOGY

*Incubation period*—Two hundred and fifty nine cases following a single exposure have shown an incubation period of 24 hours to 10 days. The period was twenty four hours in 6 per cent and 5 to 5 days in 84 per cent. The interval for the largest group (28 per cent) by days was 3 days.

The onset is sudden and prodromata have not been reported. Usual symptoms are malaise headache vertigo chilliness or definite chills general body aches and fever that may reach 103° or 104° F. accompanied by emesis sweating and prostration.

The course of the constitutional symptoms is acute lasting 2 to 3 weeks. There may be irregular fever recurring chills sweats marked prostration weakness loss of weight and possibly rhinitis or epistaxis. There is practically no apathy but stupor has been found in severe cases. The only complete records of the fever are such as have occurred in laboratory infections and they uniformly show a definite remission or intermission after the initial fever of 1 to 3 days. This break lasts 1 to 3 days and is accompanied by amelioration of the constitutional symptoms. The fever then rises to the same height as at first the symptoms reappear and

large globular masses of bacilli which are easily demonstrable and may be seen even with the objective AA as deeply staining areas. They form the most striking histological feature of the disease (Fig 175). A similar process takes place in the adrenal glands the cortical cells being chiefly involved. The glomeruli of the kidney are also infected and accumulations of mononuclear cells are found in them the lesion being not unlike that of intracapillary glomerular nephropathy in men. The essential lesion is infection of the endothelial cells, general but more marked in the vessels of certain organs. Polymorphonuclear leucocytes play but a secondary role and very rarely contain bacilli. The staining of bacilli

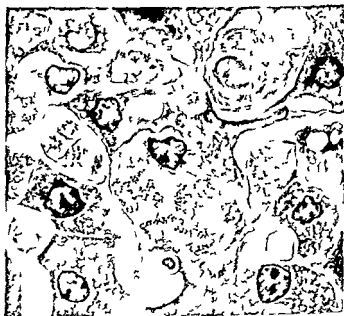


FIG. 175.—best on of liver in infection with *P. t. leuconis*

in the tissues is difficult and prolonged staining with Giemsa's solution gives the best results.

In contrast with plague the guinea pig infected with tularaemia does not show the great number of the larger organisms of *Past. pestis* which is present in stained smears and sections of the spleen and lymph glands of the plague guinea pig.

**In Man**—Verbrycke (1934) reported upon the first fatal human case in which nodules were found in the lungs and spleen. Goodpasture and House (1928) described the pathological histology of the initial lesion and they and Simpson (1928) have given a detailed study of the autopsy material.

In the acute stage an initial ulcer may occur but Ohara demonstrated by human inoculation that infection might occur without the development of such a lesion. The ulcer presents a coagulation necrosis with nuclear

Rather frequent is an eruption which is usually bilateral and not confined to any special portion of the skin. It has appeared as early as the third day and as late as the seventh week, being transient or lasting as long as 2 months. Inconstant in type it may be a blotchy erythema or be macular papular or even nodular and pustular and purpuric changes have been noted. It is usually painless and without pruritus but may be painful and inflammatory. Termination may be accompanied by desquamation or pigmentation.

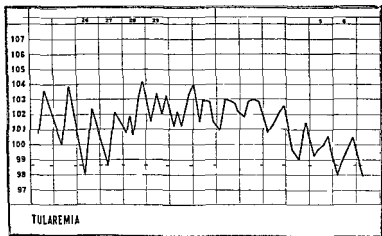


FIG 177—Temp tu h rt of a c s of laboratory nf : on (tularaem a)

**Varieties**—Francis recognizes certain varieties of tularaemia the onset and course being in general the same in all.

*Ulceroglandular tularaemia* is that which has already been described. It comprises about 84 per cent of cases. The primary lesion is a papule of the skin which later on becomes an ulcer and is accompanied by enlargement of the regional lymph glands. This type also includes cases of the *pulmonary* and *meningeal* forms nearly all of which present evidence of infection through the skin.

*Oculo glandular tularaemia* comprises about 6 per cent of cases the primary lesion being of the conjunctiva and the regional lymphadenitis being of the head and neck or possibly if severe of the axilla. It may be unilateral or bilateral and presents severe conjunctivitis with chemosis together with oedema of the lids and surrounding tissues. There is usually a papule on the lower lid and soon small discrete ulcers appear on the conjunctiva of both lids. Purulent dacryo cystitis and corneal perforations have occurred but the sinuses are unaffected. It tends to be severe and occasionally fulminating cases appear with death as early as the sixth day and presenting convulsions stupor and delirium. Herrens chnaud (1935) reports that Parinauds conjunctivitis first described in



the termination is by lysis. The pulse is rather rapid and the blood pressure is uninfluenced. The spleen is not palpable.

There is often an initial local ulceration and within 48 hours of the onset a tender painful lymphadenitis with reddened overlying skin is noticed in the glands draining the site of infection. In about half of the cases, these glands remain hard and tender for 2 to 3 months, and then slowly resolve. In the other half, they suppurate and may rupture through the skin after the acute inflammation has subsided, even after 2 years. Glands other than regional ones are occasionally involved.

About 24 hours after the discovery of the lymphadenitis a painful inflamed papule is often evident at the site of infection, which is usually on exposed parts and there may be streaks of lymphangitis. Necrosis occurs and when the core is liberated there remains a dry dark punched out ulcer about 10 millimeters in diameter. This heals slowly, and leaves a scar.



FIG. 176.—Cut section of human spleen showing nodules beneath capsule and in splenic pulp thirteen days after onset. (Tran and Callender) (Army Medical Museum No. 45152)

The blood shows a moderate (even to 16,000) leucocytosis. No agglutinins are present during the first week; they appear during the second week and there is an abrupt rise in titer during the third week, reaching its maximum (possibly 1:1280 or 1:2560) before the eighth week, when the fall begins. A gradual decrease about 1:140 is reached at the end of the first year, but specific agglutination persists for years (even for as long as 19). Simpson (1928) by means of history and agglutination tests demonstrated that tularaemia had perhaps existed unrecognized as such in Ohio for twenty years.

Subcutaneous nodules resembling sporotrichosis frequently appear (at times, in crops) on the anterior or posterior aspects of the forearm or arm and along the lymphatics between the ulcer and the regional glands. They are firm, movable, tender, four to ten millimeters or more in diameter, 1 to 30 in number, and the skin over them may be reddened. Suppuration may occur, accompanied rarely by purplish overlying skin, and they may persist as long as 7 weeks.

Sequelae are rare. Loss of vision has occurred from corneal perforation and prolapse of the iris in oculoglandular tularaemia.

Foshay found in his analysis of 518 cases that the most frequent complication was suppuration of the buboes which occurred in more than half of all cases. In 600 cases treated with serum pregnancy was a complication in 7 patients. Six women acquired the disease during pregnancy but there were no untoward occurrences and all 6 were delivered of full term healthy infants. Albuminuria was commonly observed during the febrile initial acute phase but only 2 patients showed evidences of kidney changes revealed by the urine beyond the initial albuminuria. One with normal urine at the onset developed heavy albuminuria without casts or cells in the third week of illness and died on the 36th day. The other showed urinary signs and acute nephritis during the third week but recovered. Meningitis occurred in 2 patients. In each it appeared as a late manifestation of a terminal septicaemic phase. Pleurisy occurred in 56 patients and effusions were present in 18. A pneumonic consolidation was found in 107 patients.

**Prognosis**—The disease has a mortality of about 4 per cent. Death has been reported in 24 of 650 collected cases and Foshay reports a mortality of 25 in 600 cases treated with immune serum. During the acute stage death has occurred during the typhoidal state from septicaemia and from broncho pneumonia, general peritonitis, diarrhoea and intestinal haemorrhages. Two cases terminated 3 and 5 months after the onset with albuminuria and coma. Of 6174 cases occurring in the United States 299 or 4.8 per cent have died. Francis (1937) points out that of 100 cases manifesting pulmonary complications 40 died—31 within the first month, 8 in the second month and 1 in the ninth month. Of the 40 deaths 24 presented the signs of bronchopneumonia, 7 lobar pneumonia, 1 interstitial pneumonia, 3 showed discrete multiple nodules in the lungs and 1 multiple pulmonary infarctions. Of 60 patients who recovered from pulmonary complications 16 required aspiration of pleural fluid at periods ranging from 2 weeks to 5½ months. From the chest fluid of 4 patients *Bacterium tularensis* was isolated during life 3 to 5 months after onset. Meningeal localization is usually fatal. Death resulted in 12 of 20 cases in which infection occurred by ingestion of the organism. Extensive skin ulcerations in Blackford's case yielded *B. tularensis* 5 months after onset.

Convalescence is slow, usually requiring about 4 months but a year may pass before the patient feels well again. Weakness and dyspnoea on exertion are marked and the disease may be most incapacitating. There may occasionally be mild returns of fever.

Ledingham and Fraser mention the occurrence of 3 human cases of tularaemia occurring in members of the staff of the Lister Institute who were carrying on investigations and maintaining the virus of tularaemia in animals. In 2 there was a severe degree of general weakness recurring in mild attacks and an extremely slow convalescence prolonged for over a year during which there was at least partial disability for work.

Paris, is a form of oculo glandular tularaemia. It is characterized by a granular condition of the lids with chemosis of the conjunctiva, inflammation and enlargement of the preauricular lymphatic glands. *B. tularensis* was apparently isolated from two cases.

*Glandular tularaemia* comprises about 4 per cent of cases and shows regional lymphadenitis but lacks a lesion at the site of infection.

*Typhoidal tularaemia* comprises about 5 per cent of cases, and both primary lesion and lymphadenitis are absent. Instead there is a general

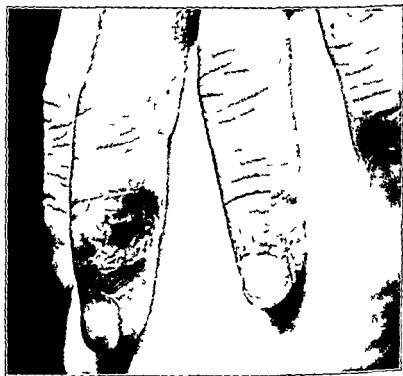


FIG. 178.—Ulcer of finger nineteen days after onset in a market man who dressed rabbits. (Brown and Hunter.)

systemic infection in which fever and prostration are the outstanding symptoms.

*Ingestion Tularaemia*—In this form the disease is contracted from eating the flesh of an infected animal insufficiently cooked.

*Sequelae and Complications*—Tularaemic septicaemia has been regarded as the most serious complication. Other complications noted especially have been acne of the back, severe herpes, pleurisy, broncho or lobar pneumonia, jaundice, meningitis and appendicitis. Three patients with the ulceroglandular type developed alarming meningeal symptoms and died. Two cases developed ascites and *Past. tularensis* was isolated from the fluid.

The cross agglutination between the immune sera of tularaemia and undulant fever and their causative organisms must be emphasized. Francis and Evans have pointed out that there is an antigenic relationship between *P. tularensis* and *Brucella abortus* and *melitensis* and that some tularaemia sera contain group agglutinins for the latter organisms. Such tularaemia sera may agglutinate *P. tularensis* more quickly and to a higher titer than they agglutinate the *Brucella*. A marked difference in titer of a patient's serum for *Past. tularensis* or for the *melitensis abortus* group reveals the diagnosis to be that of the higher titer. Practically

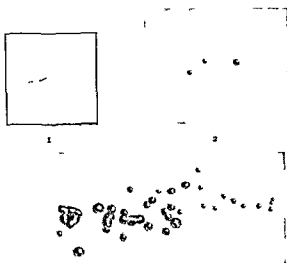


FIG. 79—1 *P. tularensis* serum ult. 2 Cult. *P. tularensis* negative 3  
day 3 Tnday aft. ult. n. fmed

equivalent titers necessitate agglutinin absorption tests. The test must be performed with great care and always with controls of normal serum, as *Past. tularensis* just as *Brucella melitensis* may show a curious tendency to spontaneous agglutination with other antigens.

In performing agglutination tests the heating of the immune serum to 55 C for 30 minutes is immaterial. It is satisfactorily preserved with an equal part of pure neutral glycerin which also clears the serum or with 0.1 per cent trikresol. The rabbit has been the animal particularly used for the development of an antiserum though Fohey has employed the goat and the horse.

In the National Institute of Health the antigen is prepared by washing off a 48 hour growth on blood glucose cystine agar with a small amount of saline containing 0.3 to 0.5 per cent formalin. The suspension is then thrown down in the centrifuge and the

Foshay in the analysis of 518 unselected cases found the typhoidal clinical type had the worst prognosis. He notes that the duration of the disease varied from a week to 15 months in most cases lasting about 4 months. Excessive suppuration of lymph nodes was the most frequent cause of protracted illness. Convalescence is usually slow. It is rare for a patient to be at work again at the end of a month, and during the third month usually only half time work is performed.

Immunity is lasting in man and may be connected with the long persistence of agglutinins. There is no record of a second attack. A local reinfection may occur, however as in the case of Francis himself. Two years after a typical attack he noted a papule on his finger and epitrochlear and axillary lymphadenitis, but without constitutional symptoms. A guinea pig was inoculated from the papule and it succumbed with typical lesions of tularaemia. The experience of Francis has been that virulent laboratory infections of guinea pigs, rabbits and white mice are uniformly fatal, with the single exception of 1 rabbit, which survived a severe acute attack. 35 days after the onset it was inoculated with a million fatal doses of a virulent culture and remained well for 21 months.

### DIAGNOSIS

When inguinal lymphadenitis is present there may be confusion clinically between venereal bubo, climatic bubo, pyogenic infection or plague. Six cases of tularaemic inguinal lymphadenitis following tick bite have occurred. Pasternack (1939) who reports 2 cases shows that clinically the disease in them might have been confused with other types of bubo.

The diagnosis also may be rendered difficult by the lack of familiarity with the disease. If tularaemia be borne in mind the combination of features is usually characteristic viz (1) a history of having dressed or dissected a wild rabbit, the bite of a tick or fly, or the handling of such insects, (2) the primary skin or conjunctival lesion, (3) the regional lymphadenitis, and (4) the fever of 2 to 3 weeks duration with its striking curve may be suggestive. The isolation of the organism either by culture or the inoculation of the guinea pig affords positive proof and the agglutination test may be of great value. Francis states there is a complete absence of agglutinins in the blood during the first week of illness but that specific agglutinins for *Bacterium tularensis* are always present at some time in the second week.

*Agglutination tests* are relied upon chiefly for diagnosis after the first week. Foshay (1940) reports agglutinins may not appear until the 3rd week or rarely until the 4th week. A titer of 1-80 or over is considered diagnostic, particularly if the titer rises as the disease progresses. Titers as high as 1-5000 have occurred, and a positive agglutination may persist for months or even years after recovery. Foshay states that once agglutinins have been acquired as a result of infection they have not yet been found to disappear completely thereafter even if tested for as long as 33 years after recovery.

in size from pin point to about one millimeter in diameter. The lungs and also the kidneys frequently show haemorrhages. In other words the gross pathological lesions resemble so closely those of infection with *Bacillus pestis* that the two infections cannot be told apart with the naked eye. However the pathological histology and the results of the bacteriological examinations are entirely different in infections with *Bacterium tularense* and *Bacillus pestis*. Thus in tularaemia infection very large numbers of a very minute round or rod shaped organism are found in microscopical preparations from the heart's blood and the liver, spleen and swollen lymphatic glands. Rarely a few bacillary larger forms are seen. These organisms stain well with carbolfuchsin, crystal violet and Giemsa's solution. They are decolorized by Gram's stain.

Cultures made from the heart's blood, the spleen and liver of guinea pigs on plain agar will in the case of *Bacterium tularense* infection reveal no growth. Cultures however made upon tubes of coagulated egg yolk or of cystine agar will reveal after 3 or 4 days in the incubator at 37° C. minute colonies which at first can only be seen by a hand lens but which may eventually attain a diameter of 1 to 2 millimeters. They appear moist translucent and drop like in character. Microscopical examination will reveal very minute round or rod shaped organisms. For the bacteriological diagnosis of *Bacillus pestis* infection in the guinea pig see p. 665.

The pathological histology of the tularaemia infection is also distinctive in the guinea pig as pointed out by Councilman and the writer.

Spleens will retain virulent organisms for at least a month if preserved in glycerin in the refrigerator but in liver they may die out.

#### PROPHYLAXIS AND TREATMENT

**Prophylaxis** --- Wild sick or dead rabbits should be handled with extreme caution. In view of the great liability to infection rubber gloves should be used by laboratory workers, marketmen, hunters, cooks, etc. when dressing or handling the carcasses of possibly infected animals. Thorough cooking destroys the infection thus rendering the flesh of the rabbit harmless. Precautions in connection with coming into contact with the insect vectors are also important.

The danger of infection of laboratory workers should be emphasized. Francis has noted 39 laboratory infections in man, all of the typhoid type which have occurred in 12 laboratories in persons who performed autopsies on infected guinea pigs, rabbits or white mice. Diagnostic and other studies involving virulent organisms and the inoculation of animals should be well segregated and only carried out in laboratories where the personnel have been carefully trained in working with dangerous diseases. Rubber gloves should be worn and animals handled almost entirely with sterilized forceps and other instruments which should be frequently sterilized in the gas flame. The carcasses of animals and infected materials should be burned and all other laboratory materials carefully sterilized both before and after the operation.

bacterial sediment is taken up in saline containing 0.3 per cent formalin. This concentrated stock suspension is diluted at the time of use with saline. This suspension may be obtained from the National Institute of Health at Washington D. C. Non virulent cultures are suitable for growing antigen.

**Intradermal Test**—Foshay has employed intracutaneous injections of a especially prepared *B. tularensis* suspension, usually about 0.05 cc. Such an injection in the case of tularaemia is said to produce a skin wheal 5 millimeters in diameter. He states that falsely positive reactions have not been observed. However of course control tests should be employed and these may be made with 0.05 cc. of physiological salt solution containing 0.05 per cent phenol. A positive reaction in undulant fever has the appearance of a positive tuberculin test and like the latter usually requires 48 hours for its development. He also employed an antiserum test injecting specific immune serum intradermally as a means of diagnosis. However, he does not recommend this for general diagnostic use since Friedewald and Hunt (1939) have not reported favorable experience with this test as a diagnostic aid.

**Isolation of the Organism**—*Past. tularensis* has not been successfully demonstrated in stained smear or section of pathological material from man so this method of diagnosis is not regarded as valuable in human infection. The organism has been isolated from the blood or glands early in the disease in a few instances but usually the results of cultivation are negative. Simpson however reported having secured cultures directly from a man. However the usual method of isolation of the organism from man has been by animal inoculation.

Guinea pigs rabbits or white mice may be inoculated with pathological material from any lesion (usually an ulcer, lymph glands blood spleen or liver). Positive results from blood have been secured as early as the third day. The pus from glands is usually negative after the first month. In the presence of pulmonary symptoms sputum may be used.

The material may be prepared by grinding in a mortar, suspension in physiological saline and straining through coarse gauze. Blood is defibrinated and mixed with an equal volume of physiological saline. Four to 8 cc. of the blood dilution may be injected intraperitoneally into a guinea pig. The suspension of other material may be injected subcutaneously over the abdomen.

The inoculation of a guinea pig is the most reliable means of diagnosis. Guinea pigs generally die after cutaneous or subcutaneous inoculation with *Bacterium tularensis* within a week, death usually occurring on the third or fourth day after inoculation. At the autopsy there is induration and thickening about the point of inoculation, subcutaneous congestion oedema and often haemorrhages of varying amount. The adjacent inguinal lymphatic glands are swollen and frequently there are haemorrhages in the region of the glands. The glands are often softened and necrotic and upon section the center is yellow and caseous in appearance. The spleen is greatly enlarged and congested and both it and the liver which is also congested contain very numerous necrotic foci which vary

of magnesium sulphate. Incision of the glands is inadvisable except in the late stages when they are liable to rupture. Then when an abscess has formed incision and drainage is advisable. The incision of nodes is not recommended. For the lesions of the eyes Vail has employed silver preparations and found them notably beneficial. A treatment that is frequently satisfactory consists of continuous hot applications of half saturated aqueous magnesium sulphate and frequent lavage of the conjunctivae with warm boric saline solution.

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*Treatment is at present largely symptomatic. Rest in bed is important and seems materially to lessen the severity of the disease. No effective drug has been discovered. Curtis (1939) has reported one case successfully treated with sulfanilamide, and Powers (1939) another case successfully treated with sulfanilamide and immune serum. Convalescent serum has not proved of value.*

Foshay has prepared an immune serum from goats and a horse by a prolonged series of subcutaneous inoculations with formaldehyde killed suspension of a virulent strain of *B. tularensis*. During the past two years he has also used horse serum in the lyophile form, dehydrated from the rapidly frozen state. An advantageous concentration of the serum was effected by simply restoring one half the water that had been previously removed.

The Mulford Biological Laboratories also supplies antitularaense horse serum in both the liquid and lyophile forms. The usual method of administration has been by the intravenous route but many patients were given it by intramuscular injection and most children were treated by subcutaneous injections. The average dose for an adult with the disease is 30 cc. The serum dosage for children below 10 years is  $\frac{1}{2}$  that for adults and from 10-14 years  $\frac{3}{4}$  that of the adult dose. If pneumonia is disclosed an additional 30 cc of the serum to the primary dose should be given at once it is stated, and whenever pneumonia is discovered the initial dose should be 60 cc administered in 30 cc doses, 24 hours apart. If marked improvement is not apparent in 72 hours, an additional 30 cc is indicated. Six hundred patients were treated with serum. 81 of the cases were observed by himself, 101 by other physicians in the Cincinnati region and 391 in other parts of the country. He compared the record in these treated cases with those of 581 untreated cases. Serum sickness occurred in 309 patients or 51.5 per cent. In most instances the severity was mild to moderate but 97 patients (16 per cent) suffered severely. An exanthem was noted in 120 patients, an incidence of 20 per cent. For the very severe forms of serum sickness he has employed histamine successfully in treatment. Horse serum has proved more toxic than goat serum. Twenty five of the treated cases died. In 6 of the cases the deaths were ascribed to heart failure. In 6 cases death occurred in patients who had no signs or symptoms of septicaemia at the time the serum was administered. Foshay states that it seems certain to all of the clinicians who saw these patients that the deaths were caused by tularaemia and were therefore chargeable to failure of the treatment. However he also states that septicaemia did not occur after serum administration in any patient who had received the optimal amount of serum.

Oosling (1939) has reported one case of the oculoglandular form successfully treated with 20 cc of this serum in 2 doses of 15 cc. There was a cutaneous reaction following its injection and later the patient developed serum sickness on the fifth day.

*Local Treatment*—For the relief of pain of the buboes Foshay believes most satisfactory a warm wet dressing saturated with an aqueous solution

and the Celebes have been reported. The Malay States, Ceylon and Netherlands India are the only countries where the infection is known to attack animals. Guinea pigs, rabbits, rats, cats and dogs are susceptible. In Colombo a cow was found to be naturally infected. In 1927 the first case was reported in a horse. This animal apparently had been regarded as refractory. It lived for 18 months after the organism had been isolated. When the horse was destroyed the organism was not isolated from its viscera and it seemed to have recovered from the infection. Nevertheless Bozell (1930) has reported the transmission of the human organism to the ass and the horse.

### ETIOLOGY AND EPIDEMIOLOGY

**Etiology**—The causative organism *Actinobacillus pseudomallei* (*Bacillus whitmorei*) is motile, its motility varying from a slow serpentine motility to quite an active one. There seem to be 2 strains, one of which grown on glycerine agar gives a wrinkled growth in 2 days and by the end of the week it is rugose and heaped up like an old growth of the tubercle bacillus. Another type produces a slimy, mucoid growth on glycerine agar and on potato the culture is similar to that described for the glanders bacillus. It liquefies gelatine and curdles milk. It produces no gas in carbohydrate media. Like *Actinobacillus* (*Pfeisterella*) *mallei* (*B. mallei*) it is gram negative and non acid fast. It grows rapidly and luxuriantly on the usual culture media, both aerobically and anaerobically but better aerobically. On the whole in culture medium it resembles *A. mallei* but it is more actively motile and liquefies gelatin more rapidly. Brown, Duncan and Henry believe that the growth in a peptonized medium containing 1 per cent sodium fumarate is of value for differentiation. In different media both organisms show at times striking polymorphism and involution forms. With Giemsa's stain it may sometimes give bipolar staining and in material from a septicæmic case one might think of plague infection. However the culture will serve to differentiate the organism from the plague bacillus.

Stanton compared the *Bacillus pseudomallei* with several strains of organisms from cases of glanders and found it agreed immunologically with certain of the strains but did not give agglutination or complement fixation reactions with others. It is suggested that *A. pseudomallei* is one of a group or a strain hitherto regarded as the bacillus of glanders. Stanton and Fletcher give the table of definition as shown on page 735.

*A. pseudomallei* is readily transferred to the guinea pig, rabbit, rat and monkey either by skin scarification, subcutaneous inoculation, feeding experiments or by nasal spray. It is usually more virulent for guinea pigs than *A. mallei*.

The Strauss reaction, the production of orchitis through inoculation of a culture intraperitoneally, is similar to that noted for glanders provided the culture is not so virulent as to kill the animal within 24 hours. If a guinea pig is infected by feeding or applying the culture to the nasal mucosa it lives about 2 weeks and shows ulcerating lesions of the nose.

## Chapter XX

# MELIOIDOSIS

### DEFINITION AND SYNONYMS

**Synonyms** —Stanton's disease glanders like disease of Rangoon

**Definition** —Meliodosis is an infectious disease closely related to glanders clinically, etiologically and pathologically. It is easily communicated to all ordinary laboratory animals but generally the horse appears not to be susceptible. The causative organism is *Actinobacillus pseudomallei* (*Bacillus whittmorei*). It appears to be a natural disease of rats and its transmission in rats and man is apparently by ingestion.

### HISTORY AND GEOGRAPHICAL DISTRIBUTION

**History** —Whittmore (1911) first noted the infection at autopsies of beggars in Rangoon and pointed out that the causative organism showed differences from that of glanders. Stanton and Fletcher (1913) encountered the same organism in an outbreak among guinea pigs and rabbits and later in rats, cats and dogs at the Kuala Lumpur laboratories of the Federated Malay States and in 1917 Stanton saw human cases of the disease in Kuala Lumpur where he isolated and identified the organism and succeeded in reproducing the disease in animals both by feeding and by inoculation. The name meliodosis (or more correctly melioidosis) was suggested by Stanton and Fletcher in order to suggest the close relationship to glanders.

**Geographical Distribution** —No cases were recognized outside of Burma and Malaya until 1927 when Pons and Advier recorded cases in Indo China. Two years later Denny and Nichols reported a case in a European in Ceylon and Mesnard and Joyeux another European case in Tonking. Stanton and Fletcher (1932) were able to find a record of 83 human cases, 38 in Burma in 1911-12, many of whom were morphine injectors, 39 in Malaya between 1917-29, 5 in French Indo China from 1925-30 and 1 in Ceylon in 1927. Forty one were Indians, 23 Burmese, 12 Chinese, 6 Europeans and 1 Annamite.

An increase in the number of cases reported in recent years is shown by the fact that between 1912 when the first report was made and 1926, there had only been noted 50 cases whereas in the next 7 years this figure was nearly doubled. By 1933, 95 had been notified, chiefly cases occurring especially in Indo China and the Netherlands Indies, 7 cases being reported in the Netherlands from 1930 to 1933. One case was also reported in Siam. With the exception of 3 cases in women and 1 infant all were male adults. Since 1933 a few more cases in Saigon, China

an infection in a rat examined for plague notwithstanding the widespread knowledge of melioidosis infection. There is no instance in the recorded cases of transfer of the infection to another case hence segregation does not

Character	Pf mallei	Pf whitmori
Morphology	Rod 1.5-3 $\mu$ long	Rod 1-2 $\mu$ long
Staining	Gram negative	Gram negative
Motility	Non motile	Motile
Conditions of growth	Aerobic	Aerobic
Broth	No pellicle no odour	Pellicle forms falls to the bottom aromatic odor
Agar slope	Only pigmented	Only metallic wrinkled pigmented
Peptone	No indol production	No indol
Gelatin	Not liquefied	Early liquefaction
White of egg	Not digested	Rapidly digested
Milk	Clot in 10 days	Clot in 4 days
Potato	Glazed yellow brown viscous later chocolate	Similar but more creamy yellow but later chocolate may become wrinkled
Pathogenicity	Weak for rodents high for Equidae and man	Weak for Equidae high for man and rodents. By experimental inoculation rodents are seen to be very susceptible by ingestion by cutaneous inoculation or via the mucosae ocular nasal buccal vaginal. The cat can be infected via the digestive tract and the disease is usually subacute in the monkey the course is more chronic. Stanton showed that sheep and goat were infectible pigs were very refractory and the ass and horse but little susceptible. Birds were also refractory.
Cutaneous reaction	Positive in glandered horses rarely positive to whitmori in glandered patients	Positive to mallein in melioidosis patients horse with melioidosis negative to mallein
Strauss reaction	Positive	Positive
Complement fixation	Positive with one type (Muktesar) which is closely allied to Pf whitmori	Positive

seem to be called for. A number of morphine injectors have been found with melioidosis but Stanton thinks this a coincidence. However if the virus was introduced by the wound of the syringe needle a type of infection similar to that seen in animals when inoculated subcutaneously

caseous deposits in the lungs, and enlarged tracheal glands. It quickly succumbs to a septicaemia (24 hours) if parenteral methods are used. The organisms is excreted in the urine and faeces of infected laboratory animals.

**Epidemiology**—Although melioidosis is a glanders like disease in human beings, it is noteworthy that horses are not associated with its origin. As pointed out the first case of melioidosis in a horse was reported

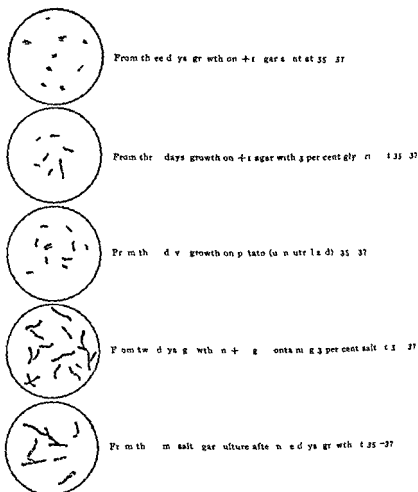


FIG. 180.—Morphology of *Actinobacillus mallei* showing polymorphism (W. B. Wherry Govt. Biol. Labs. Manila)

in 1921. The bacillus was isolated from pus from its nose and the horse's serum agglutinated *A. pseudomallei* in a dilution of 1:8000. This horse was imported into the Malay States from Australia. Melioidosis seems to be a natural disease of rats and to be transmitted to man by the ingestion of food contaminated with their urine or sputum. Although rats are supposed to be the reservoir of virus there has been no report of such

an infection in a rat examined for plague notwithstanding the widespread knowledge of melioidosis infection. There is no instance in the recorded cases of transfer of the infection to another case hence segregation does not

Character	<i>Pf mallei</i>	<i>Pf whitmori</i>
Morphology	Rod 1.5-3 $\mu$ long	Rod 1-2 $\mu$ long
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Motility	Non motile	Motile
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Milk	Clot in 10 days	Clot in 4 days
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Cutaneous reaction	Positive in glandered horses especially positive to whitmori in glandered patients	Positive to mallein in melioidosis patients horse with melioidosis negative to mallein
Serological reaction	Positive	Positive
Complement fixation	Positive with one type (Muktesari) which is closely allied to <i>Pf whitmori</i>	Positive

seem to be called for. A number of morphine injectors have been found with melioidosis but Stanton thinks this a coincidence. However if the virus was introduced by the wound of the syringe needle a type of infection similar to that seen in animals when inoculated subcutaneously

would probably result in man. In one European case, there was a history of living in a house infected with rats. Contaminated food is the probable source of human infection and the organism has been isolated in one case in man from the intestinal contents.



FIG 181—Two nodules in the lung in a case of human glanders (W. B. Wherry, Gov't Biol. Labs., Manila.)

### PATHOLOGY

Cutaneous vesicles, pustules, or abscesses of the skin or sinuses resembling glanders lesions have been reported especially in those who have been addicted to injecting morphine. In a number of instances lesions have been found in the lungs. The lesions are said to start as tubercle-like foci but to be less numerous and more scattered than miliary tubercles.

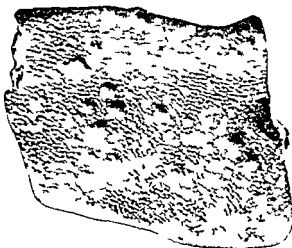


FIG 182—Cutaneous eruption in human glanders. Lack of umbilication and variation in size serve as differentiation from smallpox. (W. B. Wherry, Government Biological Laboratories, Manila.)

These nodules are made up of pus cells surrounded by a zone of congestion and when large enough to be visible they break down into abscesses containing caseous material. The nodules or abscesses next to the lungs are most common in the spleen and liver but have been found in every organ.

except the brain. Naturally infected rats may show a massive caseation of the lungs.

The subcutaneous injection and haemorrhagic enlargement of cervical and axillary glands which are sometimes evident may at first suggest plague infection in the rat but the lesions of the lungs are different.

The organism has been isolated from the blood, urine, sputum and fluid from cutaneous vesicles and other lesions of the disease.

### SYMPTOMATOLOGY

Melioidosis is said to sometimes simulate other common serious infections. In fulminating cases with vomiting, diarrhoea and collapse, cholera has been suggested and such cases may be dead in 2 or 3 days. Where early death does not occur a reaction may set in about the third day followed by a septic temperature course. In less virulent cases there is no initial collapse and the temperature may be high from the start and the patient quickly assumes a typhoid state. In 90 per cent of the cases of one series lung involvement became manifest and some of the patients were thought to have lobar pneumonia. Nodules in the liver or kidney may suppurate and suggest pyaemic or amoebic infection of the liver. Where patients last into the second week pustular eruptions of the skin or more deeply seated subcutaneous abscesses may develop and later on bone abscesses and discharging sinuses have been reported.

In 1927 a septicaemic case was reported in Saigon in which the organism was found to be more virulent than any of the strains hitherto isolated. In a case reported from Ceylon the patient showed symptoms of pleurisy with a septic type of temperature. A blood culture gave a thick pellicle growth which was thought to be a contamination. Just before the patient's death pus was withdrawn from the pleural cavity from which a culture of *A. pseudomallei* was obtained.

### PROGNOSIS

In one series of 50 cases all but 2 were fatal. One recovered after a long illness and the other was still alive after 2 years but continued to suffer with chronic discharging sinuses. Souchart and Ragot (1933) have also reported 2 cases which recovered after long illnesses of 2½ to 3 or 6 months. Manson Bahr states that most patients with the acute disease die within 10 days of the onset.

### DIAGNOSIS

The fulminating cases have sometimes suggested the diagnosis of cholera or plague. Those surviving into the second week may simulate malaria, typhoid or miliary tuberculosis. Tertiary syphilis and possibly glanders may perhaps be suggested in the pustular cases. Localized lesions of lungs, liver or kidneys may give rise to symptoms of pyaemic infections. In view of these facts the bacteriological diagnosis is the only



it and reproduced the infection by inoculations in monkeys. In 1889 he published a full account of the clinical symptoms and two years later cultivated the organism from the blood aspirated from the spleen during life. In 1893 he suggested the name of *Micrococcus melitensis* for it.

In 1897 Hughes suggested that the disease be called 'undulant fever'. During the same year, Wright and Semple made the important observation that the affection could be diagnosed by the agglutination of the microorganism with the blood serum of patients suffering from the malady.

In 1904 the Mediterranean Fever Commission appointed by the British Government with Kennedy Zammitt and Horrocks as members carried on extensive studies of the affection during the years 1904-1907 in Malta. They demonstrated that the *Micrococcus* leaves the body mainly in the urine and is then capable of existing for a long period outside the body. They also showed that the milk of many goats agglutinated *Micrococcus melitensis* and isolated the germ from both the milk and the blood of such animals. Many of the goats so infected did not appear to suffer particularly, but in a few in the later stages the goats were noticed to have an unusual degree of lassitude and to be off their food. In some a short hacking cough was noticed and they appeared to steadily lose flesh the coat also becoming thin. Zammitt (1905) pointed out that the organism might lodge in the udder, spleen and lymph nodes giving rise to an interstitial mastitis and splenic adenitis. The conclusion was reached that it was by the ingestion of such infected milk in Malta that the disease was commonly conveyed to man. Prophylactic measures based upon these discoveries brought about in a few years almost complete disappearance of the malady in Malta.

Interest in the disease in the United States was first aroused by the reports of cases of infection occurring among our soldiers after the Spanish American War. Cox and Musser and Sailer reported upon a single case in Puerto Rico in 1899 in which the diagnosis was made by the agglutination test.

In 1900 the writer first reported upon several cases occurring in our soldiers in the Philippine Islands. In the first case which resulted fatally *Micrococcus melitensis* was isolated from the spleen. Curry, in 1901 reported upon other cases in our soldiers in the Philippine Islands and in men who had returned to the United States from there. Craig in 1905 also observed cases in San Francisco among soldiers returning from service in the Philippine Islands. In addition he reported upon the occurrence in Washington D. C. of the first case of the disease to originate in this country.

Craig wrote that he was convinced that a careful study of the agglutination reaction with *Micrococcus melitensis* of many of the cases of obscure continued fever which are prevalent in this country would result in the demonstration that Malta fever is by no means a rare disease in the warmer portions of the United States and that many of the so-called anomalous cases of typhoid fever are in reality instances of infection with the organism of Malta fever.

Cases of undulant fever were next reported in this country from Texas by Gentry and Terenbaugh in 1911 and from Arizona by Yount and Looney in 1913. The outbreak in Phoenix, Arizona in 1922 again especially attracted attention to the infection in the United States.

Another item of interest in the history of undulant fever was the report of Nègre and Rénaud in 1912 upon the occurrence of a variant of *Micrococcus melitensis* originally isolated in Malta which they proposed to call *Micrococcus paramelitensis*. This organism differed in its agglutination reactions from the typical *Micrococcus melitensis* strains.

Bassett Smith Bruce and later others described cases of this paramelitensis fever in which the blood serum of the patient agglutinated this organism but not the typical *Micrococcus melitensis*. Nevertheless the clinical features of the disease were the same as in ordinary undulant fever. Séjournat in examining 490 goats most of which had been imported from Spain found 20 infected 9 with *Micrococcus melitensis* 9 with *Micrococcus paramelitensis* and with both of these organisms. Hence the recognition of both of these strains became of importance in connection with the diagnosis of the disease.

Subsequently Burnet found that para strains of the organism when suspended in physiological salt solution were agglutinated when subjected to a temperature of 80 C for two hours in an air bath. As a rule they are not agglutinated readily by specific sera. Intermediate variants of the other two species of *Brucella* later discovered have since been demonstrated and Mallmann and Gallo (1933) dissociated the rough from the smooth type.

While in earlier years it had been supposed that the goat was the only animal concerned with the infection of man with undulant fever recent investigations have shown that this is not the case. A contagious form of abortion in cattle was also distinctly recognized at the beginning of the 19th century and in Great Britain (in 1886) it was proposed in the House of Commons to include the epidemic form of abortion of cattle in the Contagious Disease Animal Act.

In 1897 Bang and Stribolt first demonstrated contagious abortion to be a distinct specific infection and isolated in pure culture the causative organism. They further induced abortion in a pregnant cow by injecting cultures of this organism into the vagina and again recovered it from the infected animal. Abortion was also produced in sheep and in mares by its inoculation. Bang's work was confirmed by Preiss in 1902 and by Nowak in 1908. In 1909 McFadyen and Stockholm showed that the infection could be diagnosed in animals by the agglutination complement fixation and abortion tests. In 1910 McNeal and Kerr isolated the specific organism from the disease in cattle in the United States and designated it as *Bacillus abortus*. The following year Schroeder and Cotton found this same bacillus in cow's milk and upon its inoculation into guinea pigs produced tubercle like lesions. They suggested that the organism might be pathogenic for man.

In 1911 Theobald Smith and Fabyan inoculated *Bacillus abortus* into guinea pigs and produced a disease from which the organism could be recovered. They also suggested the possibility of human infection with this organism and pointed out later that the udder of the cow might be a source of infection for the milk.

Later Traum Goode and Smith showed that the infectious abortion in swine was due to a similar organism which however grew more readily upon the surface of solid media.

In 1919 Alice Evans demonstrated the close relationship that exists between *Bacillus abortus* of cattle and *Micrococcus melitensis*. She found that morphologically culturally biochemically and by the simple agglutination test *Micrococcus melitensis* and *Bacillus abortus* were indistinguishable. Only by the absorption test could any differences be distinguished with different strains.

In 1920 Meyer suggested the generic name of *Brucella* to include both the organisms of undulant fever and of infectious abortion. There has been much discussion with reference to nomenclature in regard to some

it, and reproduced the infection by inoculations in monkeys. In 1889 he published a full account of the clinical symptoms and two years later cultivated the organism from the blood aspirated from the spleen during life. In 1893 he suggested the name of *Micrococcus melitensis* for it.

In 1897 Hughes suggested that the disease be called 'undulant fever'. During the same year Wright and Semple made the important observation that the affection could be diagnosed by the agglutination of the microorganism with the blood serum of patients suffering from the malady.

In 1904 the Mediterranean Fever Commission appointed by the British Government with Kennedy, Zammit and Horrocks as members carried on extensive studies of the affection during the years 1904-1907 in Malta. They demonstrated that the *Micrococcus* leaves the body mainly in the urine and is then capable of existing for a long period outside the body. They also showed that the milk of many goats agglutinated *Micrococcus melitensis* and isolated the germ from both the milk and the blood of such animals. Many of the goats so infected did not appear to suffer particularly, but in a few in the later stages the goats were noticed to have an unusual degree of lassitude and to be off their food. In some a short hacking cough was noticed and they appeared to steadily lose flesh; the coat also becoming thin. Zammit (1905) pointed out that the organism might lodge in the udder, spleen and lymph nodes giving rise to an interstitial mastitis and splenic adenitis. The conclusion was reached that it was by the ingestion of such infected milk in Malta that the disease was commonly conveyed to man. Prophylactic measures based upon these discoveries brought about in a few years almost complete disappearance of the malady in Malta.

Interest in the disease in the United States was first aroused by the reports of cases of infection occurring among our soldiers after the Spanish American War. Cox and Musser and Sailer, reported upon a single case in Puerto Rico in 1899, in which the diagnosis was made by the agglutination test.

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the Transvaal and Orange River State. It is also endemic in (German) South West Africa. In East Africa Brucellosis has recently been found to be the cause of cases of fever hitherto of obscure nature in 7 stations in Tanganyika Territory. The organism isolated approaches the Rhodesian type of *Br. abortus*.

The disease was formerly regarded as a subtropical or exotic one. In the United States few physicians were familiar with it or recognized it clinically and few laboratories were in possession of cultures of the causative organism or prepared to perform a bacteriological diagnosis of it.

In recent years no other affection has attracted wider attention from the clinician, bacteriologist and public health worker than undulant fever and it has been shown to be world wide in its prevalence as pointed out above. Indeed at a recent meeting of the Health Section of the League of Nations the statement was made that undulant fever was one of the most important problems facing public health workers.

In the United States prior to 1925 about 128 cases of undulant fever had been reported particularly from Texas, New Mexico and Arizona where there was apparently a true endemic center.

Gentry and Ferenbaugh believe that the affection which is known in Texas and New Mexico as slow fever, mountain fever or goat fever is really undulant fever and that it has existed there for 5 years prior to 1911. During the period from 1925-28 the recorded cases in the United States were 24, 46 and 217 respectively. During 1928 probably largely due to the dissemination of information regarding the disease 649 cases were recorded. In 1929 Hasseltine and Simpson stated that it was recognized in every State of the Union. During that year Hardy collected epidemiologic data on 330 cases and Hasseltine upon 109. Simpson, who conducted a survey of the number of cases of undulant fever recorded by the State Health Departments of this country during 1929 found that 301 cases occurring in every state of the Union were reported during that year and a total of 2365 cases were reported up to January 1, 1930.

In the State of Iowa alone Hardy and his colleagues studied 375 cases. This state is one in which cattle and hog raising are the two main industries and contagious abortion is prevalent in both these animals. There are very few goats and herds of sheep are scattered. These facts suggested that most of the undulant fever in the state was of bovine or porcine origin and this supposition has been confirmed by the bacteriological examinations. In Ohio in 1929-30 Simpson found and studied 90 cases of the disease in and about Dayton. Bayly 88 cases in Minnesota and Wallace 56 cases in Tacoma, Washington. One hundred and sixty-one cases of undulant fever were reported in the State of New York during the year 1930.

In 1931 over 545 cases were recognized in the United States and in 1935 1897 were reported with the highest incidence in Iowa.

### ETIOLOGY

**Bacteriology**—Brucellosis primarily affects goats, cows and hogs frequently in pregnant animals causing abortion. Secondly these infections gain entrance to man in whom the symptoms are very similar whether the infection is of goat, cow or hog origin. The disease in man has been called undulant fever on account of the successive waves of pyrexia which may extend over several months. *Brucella melitensis* has as its chief host the goat especially in Europe and in parts of the United States. In addition to man and goats it has also been isolated from the milk of infected cows in the United States, France and Italy and from aborted fetuses of sheep and goats in France, Italy and Argentina.

variations in type. The following terminology seems most satisfactory: *Brucella melitensis* Bruce, 1887, (Caprine type), *Brucella abortus* Bang 1897 (Bovine type), *Brucella suis*, Traub 1914 (Porcine type)

In 1922 Bevan on epidemiological grounds suggested that the undulant fever of Rhodesia which had recently become prevalent there was caused by *Bacillus abortus* since no goats were kept on the ranches where cases of human undulant fever were observed. He also demonstrated that the serum from such patients agglutinated *Bacillus abortus*.

In 1924 Keefer reported upon a case which occurred in Maryland in which a diagnosis of undulant fever was finally made and in which goat's milk could be entirely excluded as the cause. Eventually *Bacillus abortus* or *Brucella abortus* var *suis* was isolated from the blood of this patient and its nature verified by serum reactions performed by Evans.

Shortly afterwards Gage and Gregory, Huddleson, Carpenter and Merriam and others reported further cases in this country caused by *Bacillus abortus*. Still more recent work has shown that infection of human beings frequently occurs either with the *Bacillus abortus* of cattle or with one of its varieties. *Br. abortus* has also been encountered in the inflammatory lesions of poll evil and of fistulous withers in horses, but there is some difference of opinion as to what extent the pathological condition is due to this microorganism, as *Onchocerca cervicalis* is frequently associated with it.

### GEOGRAPHICAL DISTRIBUTION

Undulant fever, particularly associated with the use of goat's milk as food, was at first presumed to be limited to the Mediterranean region with highly infected centers at Malta and Gibraltar. Through improved bacteriological methods cases of the infection were soon detected, not only elsewhere in Europe but in other parts of the world as well, notably India, China, the Philippine Islands, South Africa, Arabia, the southwestern United States, Mexico, the West Indies, and portions of South America.

In Europe, besides the shores and islands of the Mediterranean such as Sicily, Cyprus, Candia, Sardinia, and Corsica, the disease exists in Portugal, France, Italy, the Levant, and the Balkan states. Spain, Granada, Barcelona, and Murcia have more recently been infected, while in France in 1924 the disease was found to have spread and to exist in endemo-epidemic form in the Departments of Gard, Hérault, Savoy, the Dauphiné, the Cévennes, and the Seine, where several thousand cases were recorded from 1920 to 1924 by Auché and Dubois. The Pockefeller Undulant Fever Center (1930) found that the disease was widespread in man, sheep, and goats in southeast France. It is also prevalent in Germany, Denmark, Sweden, and Switzerland. Madson (1940) reports that in Denmark between 1927 and 1924 the serum reactions of the Copenhagen Institute revealed an evidence of Brucellosis reaching 40 per 100,000 for males between 25 and 35 years of age.

In the Far East it is endemic in northern India, in the Punjab, with occasional cases in Bombay and Ceylon. It has occurred in China, particularly in the Yangtze region, in the Philippine Islands, Dutch East Indies, and Australia, at Aden and Suakim, and in Trans-Caucasia.

In South America it has been observed from Venezuela, Chile, Brazil, Argentina, Uruguay, Cuba, and Porto Rico, and there is probably a small center of infection in Peru.

In Africa it is reported from Egypt, Tunis, Algiers, Morocco, the Sudan, Blue Nile, Lake Chad, and Uganda. During the Boer War it became prevalent in South Africa.

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The three organisms usually occur singly or in pairs but when grown in bouillon often appear in short chains. They are non motile, possess no capsule and produce no spores and are Gram negative. In hanging-drop preparations brownian movement is usually marked.

**Cultural Characteristics**—The organisms grow very slowly on ordinary laboratory media but better on beef or liver infusion agar or broth adjusted to a pH of 6.8 to 7.4 and incubated at 37° C. In primary cultures the colonies become visible in 3 to 6 days and are small dew-drop-like later becoming opaque and raised. In broth there is a diffuse turbidity.

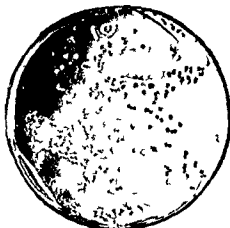


FIG 184.—Colonies of *Brucella abortus* from infected milk. (After Huddleson, Curtiss, C. M. M. W. with Fund. N. W. York.)

The organisms are killed by heating to 60° C. The optimum temperature of growth is about 37° C. At temperatures over 40° the growth is suspended and above 42° artificial cultures die. *Brucella melitensis*, the caprine variety, is aerobic but may also grow more slowly anaerobically.

One of the most striking features in regard to cultivation is the slow growth. Cultures made directly from organs after death on agar may show colonies after 48 to 72 hours but others show no growth for 4 or 5 days even if kept at 37° C. and no growth for 7 days if kept at 25° C.

Observers have sometimes discarded or thrown away cultures before they had time to develop. The colonies when they appear upon the surface of the media are smooth and transparent resembling dew-drops. Under the microscope they are round or oval and are transparent with an even border and with a slight brown tinge about the center and are finely granular. As they grow older they become darker in color. Gelatin is not liquefied. On this medium the colonies are hardly perceptible at the end of the first week. In beef broth growth is rarely apparent earlier than the fourth day and occasionally not until 10 days. The cultures first become cloudy and the growth



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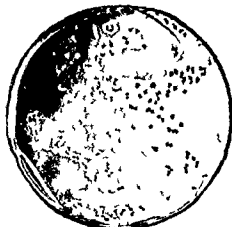


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appears more luxuriant in the upper layers. Still later a white precipitate may form at the bottom in which the organism occurs in short chains. In neutral litmus milk, slight development is sometimes observed in 48 hours. The alkaline reaction continues to increase. There is no coagulation. In glucose peptone 1 per cent lactose saccharose and starch peptone no acid or gas is produced. No indol is formed in the cultures. The addition of 1 per cent nutrose with some strains quickens the growth and when it is desirable to differentiate the organism from certain other bacteria it is of advantage to use a medium containing glucose nutrose and litmus. The fact that *Brucella melitensis* does not ferment glucose and renders milk and other media alkaline is an important feature. With such a glucose nutrose or litmus agar the colonies appear blue whereas many of the streptococci or bacilli found in the urine faeces etc ferment glucose and give an acid reaction.

**Differential Characters**—The similarity of the 3 species has stimulated efforts to discover differences which would serve to identify a species irrespective of the host from which it was isolated. Differences have been sought particularly in carbon dioxide requirements serological relationships bacteriostatic action of dyes hydrogen sulphide production and glucose utilization.

**Increased Carbon Dioxide Tension**—*Br. abortus* was originally isolated by Bang who inoculated material from the uterus of an aborting cow into deep tubes of serum agar in which colonies developed only in a zone of partial oxygen tension 1 cm. below the surface. Huddleson found that atmospheric air containing approximately 10 per cent  $\text{CO}_2$  was suitable for original isolation of *Br. abortus*. After freshly isolated cultures of *Br. abortus* have been subcultured about 10 times they become adapted to grow freely thereafter in ordinary atmospheric air and the  $\text{CO}_2$  requirement cannot be restored to them by long residence in an artificially infected animal. Furthermore a recently isolated *Br. abortus* culture cannot be made to lose its  $\text{CO}_2$  requirement of isolation by long residence in a goat which is the normal host of the air growing *Br. melitensis*. Since normal strains of *Br. abortus* cannot be isolated originally in atmospheric air but only under increased  $\text{CO}_2$  tension the important differential character is established that a *Brucella* organism which requires carbon dioxide for original isolation is *Br. abortus*. There are however strains of *Br. abortus* in cattle and man in Rhodesia which grow freely in normal air from the start but the question arises as to whether they may not be vaccinal strains derived from living vaccine used for protecting cattle against contagious abortion. *B. melitensis* and *Br. suis* do not require  $\text{CO}_2$  for original isolation or for subsequent growth although both species are susceptible of isolation in 10 per cent  $\text{CO}_2$  and of subcultivation under increased carbon dioxide tension. *Br. suis* grows more rapidly if the culture in the liquid media is incubated in  $\text{CO}_2$  at a tension of 25 per cent above that of atmospheric air.

**Serological Relationships**—The 3 species are agglutinated equally by an antiserum prepared from any one of the 3 therefore the species cannot be differentiated by simple agglutination tests. *Br. abortus* cannot be differentiated from *Br. suis* by agglutinin absorption tests but *Br. abortus* or *Br. suis* on the other hand can in most instances be differentiated from *Br. melitensis* by agglutinin absorption.

However there still remains a large number of *Br. abortus* cultures which cannot be distinguished by agglutinin absorption from *Br. melitensis*. The conclusion is that agglutinin absorption is a very unreliable procedure for differentiation of the 3 species. Tularaemia sera agglutinate the *Brucella* oftener than brucellosis sera agglutinate *P. tularensis* but usually a correct differentiation between genera can be made by agglutinin absorption.

**Bacteriostatic Action of Dyes**—Huddleson and Abell have been able to separate the 3 types by the inhibitory effect on growth which certain dyes exercise when incorporated into a culture medium of beef liver agar as shown by the following table

	Methyl violet 1 100 000	Basic fuchsin 1 25 000	Pyronin 1 200 000	Thionin 1 50 000
<i>Br suis</i>	No growth	No growth	No growth	Growth
<i>Br abortus</i>	Growth	Growth	Growth	No growth
<i>Br melitensis</i>	Growth	Growth	Growth	Growth

However occasionally some strains of *Br abortus* will not develop in media containing any of these dyes

**Hydrogen Sulphide Production**.—Huddleson Hasley and Torrey have shown that depending upon the amounts of available sulphur in the culture medium *Br suis* produces hydrogen sulphide for the longest time *Br abortus* less and *Br melitensis* not at all. The test is made by suspending a strip of dry lead acetate paper in the culture tube above the liver infusion agar at the time of inoculation and observing the degree of darkening during four days

The Danish strain of *Br suis* is reported to be different. Knutsen and Thomsen (1931) found that the Danish strain of *Br suis* differs from those isolated in the United States in that it produces little if any H<sub>2</sub>S when grown on a suitable solid culture medium

**Glucose Utilization**—It has been mentioned that *Brucella* does not give rise to acid production in glucose as tested by the usual qualitative methods in fermentation tubes with a proper indicator. However McAlpine and Slanetz have found that when the actual sugar is estimated after 7 days growth in 1 per cent glucose peptone water *Brucella melitensis* and *Brucella abortus* of the porcine type are found to have utilized at least 4 to 18 per cent of the sugar whereas the bovine *abortus* type has not affected more than 2 per cent

They believed that this glucose utilization test might serve as a valuable method of differentiation of the different races. However Meyer and Eddie failed to obtain clear-cut results with this test and it has been found that after cultivation for some time in the laboratory both the *melitensis* and porcine *abortus* types tend to lose their power of utilizing glucose and in this respect are then no longer differentiable from the bovine type. McAlpine, Plastringe and Brigham also confirmed this fact. Soule believes that both the bovine and porcine strains utilize glucose. Coleman reported that porcine strains do not ferment monosaccharides while the bovine strains do. However Mallardo, Zobel and Meyer have found that all 3 of the races *Brucella melitensis*, *Brucella abortus* and *Brucella parameitensis* after 21 days cultivation have the same fermentative powers on various sugar media whether grown alone or in symbiosis with other micro-organisms

Ohtzki and Bromberg from the examination of relatively few strains believe that *Brucella melitensis* requires a higher concentration of peptone and of phosphate for growth than does *Brucella abortus* and that it is more susceptible to higher salt concentrations particularly of calcium chloride. By the use of media with different concentrations of peptone, phosphate and other salts they believe they are able to distinguish between *Brucella melitensis* and *Brucella abortus*. They find that the

amount of glucose used depends on the concentration of peptone in the medium. With low concentrations of peptone such as 0.2 per cent *Brucella abortus* is able to utilize a considerable amount of glucose, whereas with 1 per cent peptone no glucose is used.

**Reduction of Nitrates and Nitrites**—Zobell and Meyer (1932), have reported a sufficient difference in the nitrate and nitrite reducing ability of the 3 species of *Brucella* to aid in distinguishing them.

The presence of nitrites, disappearance of nitrates and evolution of gas were observed as criteria of nitrate reduction. On the addition of 0.2 per cent potassium nitrite to a semisolid medium containing agar peptone and beef extract the *abortus* and the *suis* types grow dispersed throughout the medium demonstrating an appreciable pseudoanaerobic growth but the growth of *melitensis* strains localizes a few millimeters from the surface. The *suis* strain was found to destroy 0.05 per cent potassium nitrite in 5 days while the *abortus* and *melitensis* types lack this ability. The *melitensis* varieties are in general more active reducers of nitrites than the *abortus*.

In mediums containing 0.2 per cent each of potassium nitrate and potassium iodide the *suis* types evolve an abundance of nitrogen gas with a rapid disappearance of nitrates and nitrites while the *abortus* types very rarely liberate gas under identical conditions. The *melitensis* and the Danish porcine strains which exhibit no pseudo anaerobic growth although capable of destroying 0.002 per cent potassium nitrite fail to generate gas.

**Differences in Pathogenicity for Animals**—Monkeys, when inoculated subcutaneously or intravenously with a virulent strain of *Brucella* or fed with such cultures frequently develop febrile attacks of the disease. The British Commission fed goat's milk containing *Brucella melitensis* to monkeys with the result that 83 per cent of the animals became infected. The writer had no difficulty in infecting monkeys (*Pithecius philippinensis*) by subcutaneous inoculation with *Brucella melitensis* and of later recovering the organism from the spleen and blood of such animals. Burnet was unable to infect either monkeys or man by subcutaneous inoculation with *Brucella abortus* although controls were infected readily with *Brucella melitensis*.

The more recent work of Huddleson and Hallman and Meyer and Eddie also would appear to show that *Brucella abortus* of the bovine type has only a low infective power while the *melitensis* and particularly the porcine *abortus* type are highly infective for these animals and set up the disease usually even after oral administration. In Huddleson's and Hallman's experiments one strain of *Brucella abortus* of porcine origin (*Brucella suis*) appeared to be even more virulent for monkeys than a strain of *Brucella melitensis*.

With reference to the behavior of guinea pigs to the different strains opinions are somewhat at variance. Fyre found that by a series of intracerebral inoculations comprising rapid passages from guinea pig to guinea pig the virulence of *Brucella melitensis* can be exalted to a high pitch for this particular animal. Some observers have found that the *melitensis* strain appears to be more toxic than that of bovine *abortus*.

Subcutaneous inoculations of guinea pigs would seem to indicate that the porcine *abortus* is generally the most virulent and *melitensis* the least virulent for these animals. Hardy in the study of two varieties of *Brucella abortus* found that one a porcine strain gave very marked lesions in guinea pigs while the second a bovine one had little effect on these animals.

Of 23 strains of human origin which came into Theobald Smith's hands 3 had no effect whatever on guinea pigs. He remarked that the

entirely negative outcome of inoculation into guinea pigs with infected tissue or cultures even in high dilutions has not occurred in the hands of bovine cases in his experience. One caprine strain studied by him was entirely innocuous to guinea pigs and not even recoverable from the spleen and he remarked that the absence of virulence in this instance might be ascribed to prolonged artificial cultivation. In general he believed that porcine strains produce more marked lesions in guinea pigs which tend to suppurate than do bovine ones.

Meyer and Eddie state that some strains of caprine *melitensis* may give the same results and be as virulent in guinea pigs as porcine strains while some porcine strains may show little or no pathogenicity for guinea pigs. The *abortus* variety was more in some and virulent as a rule than the *melitensis*. Jaffé found that the inflammatory changes produced by the *abortus* and *melitensis* varieties in guinea pigs were quantitatively alike with much more pronounced changes in the case of *abortus* infections. Burnet also thinks that the *abortus* variety is more virulent than the *melitensis* variety for guinea pigs. Kristensen found that the Danish porcine strains appeared to be less virulent for guinea pigs than the American ones.

Rabbits are sometimes susceptible to subcutaneous and intravenous intraperitoneal and intracerebral injection with *Brucella* although the inoculations frequently fail. The rabbit may show antibodies in its serum but rarely a septicæmia. Sometimes the only manifestation of infection is progressive emaciation.

Cantani in his early experiments considered that he had established a marked difference between *Brucella melitensis* and *Brucella abortus* as regards the pathogenicity for rabbits. However in his later researches in which he also injected suspensions of these microorganisms intracranially he has been unable to confirm his former conclusions. Obviously the separation of the different strains or races according to their pathogenicity has not given entirely definite results. In making such comparisons it is important to work with freshly isolated strains inoculated under comparable conditions.

**Lesions in Guinea Pigs.**—Theobald Smith points out that the infection of *abortus* in guinea pigs is non-fatal and self-limited. Whether the inoculation is made subcutaneously or intraperitoneally and whether the dose is large or small the condition of the animal at about eight weeks is the same. No local lesion is produced or if some swelling occurs it disappears. The regional and other subcutaneous nodules may be swollen to twice their size or but little. Necrotic foci are absent unless otherwise introduced in the pathological material.

The only other changes were the large highly congested spleen from 3 to 4 times normal size with or without numerous minute gray foci and an infiltration followed by suppuration of the epididymis of one or both testicles. Microscopic lesions of the female genital organs are exceedingly rare. Rarely swelling of the carpal joints is present. The spleen contains the large number of microorganisms although *Brucella abortus* may be obtained from most other organs if sufficiently large pieces are cultured. Just beneath the capsule and sometimes deeper in the liver tissue small gray nodules may also be found.

In the study of the tissues of inoculated guinea pigs no invasion and multiplication of the organism within the epithelial cells was observed. Such a condition appears to be limited to the bovine chorionic membrane. In the guinea pigs the lesions wherever found consist of a diffuse multiplication of local reticular endothelial cells or of infiltration of mobile cells or both combined. There may be a diffuse general replacement of the normal lymphocytes by the larger monocytic type of cell which is found not only in the spleen but in the lymph nodes and interstitial tissue of the epididymis. In guinea pigs inoculated with the porcine strain the lesions were found to be more conspicuous and subject to softening and abscess formation in the spleen lymph nodes testicles and limbs.

Hardy found that guinea pigs infected with the porcine strain lose weight appear rough and not infrequently die whereas those inoculated with the bovine *abortus* strain

often appear quite healthy and may gain weight. Generally there was marked enlargement of the spleen, liver and lymph glands, usually with abscess formation and involvement of the joints, bones and testes. Tubercle formation was sometimes noted in the liver.

**Rough and Smooth Types**—The rough form of *Brucella* has been dissociated from the smooth form by Mallmann and Gallo (1933). They report that a colony of the extreme rough form is characterized by a very irregular contour while its surface is actively wrinkled. Morphologically the organisms of the rough form are from 3 to 5 times longer than in the smooth form. The long rods are granular and tend to produce chains. All 3 species of *Brucella* when rough lose their differential characteristics when grown on media containing either basic fuchsin or thionin. Huddleson found the rough form only slightly antigenic and nonpathogenic for guinea pigs. He believes that the most satisfactory and delicate method for the detection of intermediate variant forms of *Brucella* may be obtained from using citrated whole blood of non-immune or non-infected individuals for 30 minutes at 37°C. Bacteria from a smooth culture of *Brucella* are phagocytized only to a slight degree if at all by neutrophils in normal blood while if a partially dissociated strain is used the neutrophils ingest the bacteria in varying numbers.

**Vitality**—*Brucella melitensis* is somewhat resistant outside the body, withstanding desiccation from 60 to 80 days. It also will live in tap or sea water for about a month and has been found to survive in a dry condition in dust and clothing for a period of from 2 to 3 months. However it is readily killed by exposure to sunlight. Cultures of the organism kept upon agar usually gradually lose their virulence for animals. In urine which has become markedly alkaline the organism has been found alive after 6 days and in acid urine for as long as 16 days. A solution of 1 per cent carbolic acid, 4 per cent formalin or bichloride of mercury 1:2000 will destroy it in 5 to 15 minutes. *Brucella abortus* may remain viable in moist manure for 75 days. Violle points out that in cheese, particularly Roquefort, it can remain alive on an average of 2 months. Carpenter and Boak found that when cream and butter were inoculated with this organism and stored at 8°C it remained viable for 10 days in cream and for 142 days in butter. The percentage of butter fat present seemed to be an important factor influencing the vitality, since with the higher percentage of butter fat the vitality of the organisms was prolonged. It is the belief of Carpenter and Boak that the germs were killed by the lactic acid which is produced in the cream.

The organisms are killed at moist heat at a temperature of 60°C in 10 to 15 minutes. The thermal death point fixed by Dalton and Eyre was 57.5°C.

**Cultures Isolated from Man in the United States**—Human cases of brucellosis have been reported from all of the states, some 1800 being reported annually in recent years by state departments of health principally on the basis of positive agglutination tests.

*Br. melitensis* has been isolated almost exclusively from residents of Texas, New Mexico, Arizona or California where goat raising is a prominent industry.

*Br. abortus* because of its CO<sub>2</sub> requirement for isolation has doubtless been missed in the laboratories in which CO<sub>2</sub> apparatus is not a part of the routine equipment used for blood cultures. Gilbert and Coleman working in the New York state laboratory incubated duplicate samples of patient's blood and cow's milk in normal air and in an atmosphere containing 5 to 10 per cent of carbon dioxide. Of 33 cultures isolated (44 from human blood and 44 from cow's milk) all showed primary growth in the CO<sub>2</sub>.

environment while practically none gave primary growth in normal air thus indicating that only the bovine (*abortus*) type is prevalent in that state where few hogs are raised. Huddleson isolated 57 cultures from man in Michigan only 2 of which were *Br suis*. The small number of the *e* corresponds to the small *e* tent of the hog raising industry in Michigan.

*Br suis* was the type in 69 of 104 cultures isolated from man in Iowa, 35 being *Br abortus*. All were isolated by Hardy, Borts and Jordan in the State laboratory where duplicate samples were incubated in air and in CO<sub>2</sub>. The high percentage of *Br suis* cultures corresponds to the prominence of the hog raising industry in Iowa. Beattie and Rice reported a milk borne epidemic of 30 cases in Council Bluffs, Iowa, confined to city users of raw milk supplied by one dairyman. *Br suis* was cultured directly in normal air from the blood of 6 of the 30 patients and obtained from cream of the milk of one cow in the dairy by guinea pig inoculation. This cow before purchase had associated with aborting hogs on another farm. *Br suis* was the classification of 32 of 33 cultures isolated by Sellers of the Georgia State laboratory from human blood which were all incubated in normal air, none in CO<sub>2</sub> because of the lack of suitable apparatus. Whether the failure to isolate *Br abortus* was due to the failure to use CO<sub>2</sub> apparatus remains a question. The epidemiological evidence, however, points to drinking raw cow's milk containing *Br suis* as the cause. Evidence of the spread of *Br abortus* from cows to hogs by contact is lacking, but the spread of *Br suis* from hogs to cows by contact is amply demonstrated and man may be infected by drinking the milk of such cows.

As the porcine strain is more invasive for man than the bovine, the infection may be transferred by milk containing a relatively small number of organisms, whereas milk heavily contaminated by the bovine strain may not transmit the infection. Of the organisms isolated from the blood abscesses, urine and faeces during 1935 in the United States, 70 to 93 per cent were of the porcine type and the remainder of the bovine type, except for an occasional caprine strain.

## EPIDEMIOLOGY

**Manner of Infection.**—The disease may be contracted by drinking raw milk from diseased goats or cows, by contact with infected animals, or by handling infected meat, also by eating infected cheese and butter, since the souring of milk often does not always destroy the organism. Fifty-seven laboratory infections have been recorded in 17 laboratories in the United States. Infection may occur through the skin, as well as through the alimentary tract. The spread of the disease through carriers has not been demonstrated, though the organism is present in the urine in about 10 per cent of the cases and has been reported in a few instances in the faeces. It has also been found in human milk. Cases occur at all ages, except usually during the nursing period of infants. Nursing children have sometimes been infected.

Orr and Huddleson found in Michigan that in a group of 500 individuals equally divided into males and females of all age groups, constantly exposed to the *abortus* organism through an infected milk supply, only 1.4 per cent gave evidence of infection with this organism, and only 0.8 per cent showed any signs of active infection. Evidence of a very low rate of human infection in localities where there is a high rate of bovine infection with *Brucella abortus* also has been obtained by other investigators in the United States and other countries (Europe, Africa and South America). Hence it would appear that in cow's milk only the most virulent strains of *Brucella abortus* may cause undulant fever in man and perhaps only

in particularly susceptible individuals. In its relationship to infection of man *Brucella abortus* may stand to *Brucella melitensis* in a somewhat similar relationship as does the bovine to the human tubercle bacillus.

While the commonest source of infection of man would appear to be milk, Violle emphasizes particularly the danger of infected Roquefort cheese in France, in which the organism may be viable for as long as two months. Huddleson found that *Brucella abortus* is especially present in cream. Man particularly in slaughter houses may also acquire the disease by contact with infected tissues or discharges of animals and aborting cattle, hogs, goats, or sheep may be a source of danger.

In Europe different investigators have emphasized the high rate of infection with undulant fever among men employed in slaughter houses. Dubois and Sollier found that of 480 cases of undulant fever studied in southern France seven eighths occurred in persons coming in contact with infected animals, chiefly shepherds, farmers and farm laborers, butchers and other slaughter house workers.

Of the remaining one eighth several were in laboratory workers who had handled cultures of *Brucella abortus* and *Brucella melitensis* and infected blood. They considered undulant fever definitely to be an occupational disease.

Netter believes that the organism from cattle as a rule infects through cutaneous abrasions in persons having contact with cattle suffering from epidemic abortion and that hence farmers and even more dairy men and veterinarians who take care of sick cattle are the ordinary victims much more than persons who have drunk milk from sick cows. Therefore all persons who come in contact with sick cows should wear gloves.

Hardy in Iowa also concludes that approximately one half of the cases result from contact with infected animals, their tissues and discharges, the infection in all probability entering through the skin. He points out the significance of contact with hogs in the group of packing house workers, particularly those on the killing floor who are intimately exposed to fresh carcasses where meat axe and knife lay bare the porcine organisms in the infected tissues which are afforded direct access through the skin of the worker, the skin being so often cut or abraded through his occupation. Farmers are also frequently exposed to infection through contact with hogs as in vaccinating, castrating and medicating struggling animals and in loading the animals for market. A special type of contact occurs in the manual removal of placentas. Veterinarians not infrequently become infected in this way. Habs has recently reported 5 cases in veterinary surgeons in Germany where the disease was believed to have been contracted by direct infection from the removal of placentas of cattle through cuts or abrasions on the fingers. Huddleson and Johnson found that the serum in 57 per cent of 49 practicing veterinarians gave a positive agglutinating reaction with *Brucella*. Other cases have been reported where the infection has been acquired from aborting goats, sheep and hogs.

Apparently the cow is a greater source of infection at the time of the abortion and for two or three weeks following, the foetal membranes (chorion), foetus and uterine discharges and udder ducts all containing the *Brucella*. The uterine discharges of the infected cow at full term also contain numerous organisms. It is said that such cows may act as carriers of the infection for many years. Bulls also may carry the disease particularly in the seminal vesicles. *Brucella abortus* has seldom been demonstrated in the blood of cattle and for some reason not understood the cow unlike the goat does not appear to excrete the organism in the urine.

*Brucella abortus* is also usually excreted in the milk of infected cows in relatively smaller numbers than is *Brucella melitensis* in the milk of goats. Nevertheless infections in man have been said to occur from milking both infected cows and goats. In southern France where the disease has recently become alarmingly prevalent the infection of a number of cases has been attributed to contact with ewes which have been known to excrete the organism in the urine.

**Water borne Outbreak**—Newitt and his colleagues (1938) have reported a remarkable outbreak of 80 cases of infection with *Brucella melitensis* with 1 death which occurred at Michigan State College. All the patients were students or others using a bacteriology building housing a laboratory that handled large numbers of *Brucella* cultures.

There were 20 students enrolled for laboratory courses in bacteriology who worked in the laboratories of the building. Frank clinical illness was present in 37 cases and 23 were latent or subclinical. The attack rate varied with each of the 8 bacteriology classes but of a total of 250 exposures the rate was 30.9 per cent. In addition to 65 cases in the regular classes 5 other cases occurred. Every person found to be infected with *Brucella melitensis* had been in the bacteriology building. None of the patients had had any contact with goats or goat's milk. All evidence pointed to faulty plumbing as the source of infection. By opening several faucets in the basement a negative pressure was produced in the faucet at the sink at which the glassware from the *Brucella* laboratory was washed and the water supply of the laboratory thus infected.

**Man as a Source of Infection**—The undulant fever patient may occasionally be a source of infection. The urine would appear to be the chief path by which the organism leaves the body. In some instances the *Brucella* organisms are scanty while in others they are most numerous.

Kennedy found them ranging from 3 or 4 to 30 or 40 per cc. In some 300 observations made upon man he found *Brucella melitensis* in 10 per cent. The excretion in the urine may go on for a long time even two years after the patient's convalescing. Shaw examined 525 stock yard laborers and found that 79.05 per cent gave a distinct agglutination with *Brucella melitensis*. In 7 of these the organism was isolated from the urine. Two were kept under observation and continued to pass large numbers of the organism in the urine for more than a year. *Brucella abortus* has also been recovered from the urine in some cases. In 8 instances reported by Kerr in which cultures from the urine were made *Brucella abortus* was recovered from 2.

Bruce noted that although the microorganism probably also passes out of the body by way of the alimentary tract it had only been directly observed in the faeces of man on one occasion. In this instance Eyre made plate cultures from the light colored faeces from the colon at autopsy of a case of undulant fever and obtained a mixed growth of a large variety of bacteria including *Micrococcus melitensis*. By emulsifying some of the growth in saline solution and precipitating the microorganism by the addition of a powerful agglutinating serum and again plating the sedimented micrococci he was able to obtain the organism in pure culture. Eyre reported micrococci throughout the whole length of the intestine in artificially inoculated rabbits. In addition he succeeded in isolating *Brucella melitensis* from the intestinal mucus and faecal masses in inoculated guinea pigs in infections of an acute type.

Recently Amoss and Poston have been more successful in isolating *Brucella* from the faeces of man by means of a special technique. The stool suspension was treated with immune serum to clump any organisms present and the sediment produced by differential centrifugation was seeded onto Teague medium.



Two plates were then inoculated in air and 2 in 10 per cent CO<sub>2</sub>. By this method organisms of the *Brucella* group were obtained 78 times from the stools of 6 patients. In 1 of the 6 *Brucella* was isolated from the fluid obtained by duodenal drainage before operation and from the gall bladder contents at operation.

Horrocks and Kennedy and Bull and Gram had also found *Brucella melitensis* in the gall bladder of man. Otero and Dooley likewise recorded the isolation of *Brucella* from the stools each in one instance. While there is little evidence that *Brucella* infection is spread from man to man by the organisms eliminated by the stools the method of isolation suggested by Amoss and Poston may give further epidemiological data upon this point.

Another path by which the organism may leave the body is in the milk. In 3 infected women who were examined by the British Commission *Brucella melitensis* was recovered from the milk of 2 of them. While nursing children have sometimes been infected by their mothers this usually does not occur probably because the child is gradually immunized against infection.

Some individuals acquire infection from direct contact with the sick probably from soiled bed linen or urine through hand to mouth infection. Dargain and Plazey report an outbreak of 7 cases of undulant fever among a group of 14 men who lived together. The infection was believed to be traced to a pet dog with 3 puppies 2 of which were born dead.

Since the organism may frequently be isolated from the blood the possibility of infected mosquitoes or other biting insects must be considered. Although Horrocks and Kennedy reported the presence of *Brucella melitensis* 4 times in 275 specimens of *Culex fatigans* and *Aedes fasciatus* caught in wards where cases of undulant fever were being treated and successfully infected monkeys with material inoculated from these mosquitoes these results have not been since confirmed and there is no evidence that insects play any part in the transmission of the disease. Wollmann found that flies which had been placed in contact with cultures of *Brucella abortus* could up to 24 hours convey this organism directly to other cultures but not after that time apparently auto sterilization in the flies having taken place.

The danger of nursing patients particularly when abrasions have been present on the hands must be recognized and it is obvious that direct infection may occur in this manner. Manson Bahr in pointing out that while undulant fever is not generally transmitted directly from the sick to the well person says that it is a very striking circumstance that in some hospitals the nurses and attendants in the fever wards are ten times more liable to contract the disease than people not so employed.

Vielle calls the disease both infectious and contagious and points to the danger of infection from the excretions in which the organisms are eliminated irregularly but at times in large quantities and over a period of years.

**Carriers**—Little evidence of the spread of undulant fever through human carriers has been presented though the British Commission found that the excretion of the organism may go on for as long a time as 2 years after the patient was convalescent. Shaw isolated the organism from the urine and blood of 3 apparently healthy stock yard laborers, and Huddleson and Johnson, Giordano and Ableson and Wallace have given other evidence of infection in perfectly normal individuals. Hence the spread of the disease by carriers would seem to be possible.

**Portals of Entry**—In the great majority of instances undulant fever infection is contracted through the alimentary tract either by infected milk or dairy products butter cheese or ice cream. The organism is able to invade the normal mucous membrane of the alimentary canal and thus gain access to the circulation. Infection may also occur through other

mucous membranes notably the normal conjunctiva. The writer has knowledge of a human case in which infection from a culture was definitely proved to have occurred in this manner. Shaw was able to infect monkeys by placing a suspension of *Brucella melitensis* in the conjunctival sac and Schroeder and Cotton were able to infect a heifer by placing a single drop of *Brucella abortus* culture in the eye. Infection may also occur through the normal nasal passages pharynx interior of the larynx and trachea as has been demonstrated by experiments on monkeys. The British Commission was also able to infect monkeys through the unbroken mucous membrane of the glans penis. Since *Brucella melitensis* had been isolated from vaginal swabbings of infected women they pointed out the possibility of infection of man by sexual intercourse.

**Infection through the Skin**—Recent experimental work and many observations have shown that infection through the skin is not infrequent probably through cuts or small unnoticed abrasions. Hardy believes that in a large proportion of his cases infections resulted from direct contact and even through normal skin.

He performed with Hudson and Jordan experiments upon guinea pigs in which two varieties of *Brucella abortus* were used both isolated from human cases. One was identified as a porcine strain and the second as one of bovine origin. In one group of guinea pigs an area of the skin was shaved and abraded. In another the skin was shaved without abrasions and in another the hair was merely clipped. The infecting dose was applied by means of a glass rod. It was found that guinea pigs which did not show agglutinins in their blood if killed subsequently showed no evidence of infection. Of the guinea pigs with abraded skin 100 per cent were infected in both series. In those with shaved skin but not abraded 95 per cent were infected by the porcine strain and 82 per cent by the bovine. In those in which hair was only clipped 81 per cent porcine and 73 per cent bovine were infected. In a second series of experiments in which the guinea pigs were fed by the mouth with infective material 17 per cent of the porcine series were infected and 33 per cent of the bovine. It was thus shown that infection of the guinea pig was more readily obtained through the skin than by the oral route.

These experiments are of considerable importance but whether infection may occur with *Brucella* through the entirely normal skin seems still questionable. There are possible sources of error in the use of guinea pigs in such experiments particularly in the removal of the hair.

Some investigators doubt that infection in man is likely to occur through the normal skin. On the other hand it is quite obvious that infection may readily occur through punctures or through small wounds or abrasions of the skin unnoticed when the individual has been in contact with infected material. The numerous observations of Cesari Aublant Dubois Hardy Netter and others are all confirmatory of this fact.

Laboratory infections with *Brucella melitensis* among bacteriologists have not been very uncommon. In at least 8 recorded instances infection by subcutaneous inoculation or by feeding cultures have occurred which was followed by characteristic symptoms after an incubation period of from 5 to about 17 days. Srodowski reports that 6 of the laboratory staff in Caucasia became infected with the disease in the last 4 years. Simpson reports that 5 workers in the Hygienic Laboratory of the United States Public Health Service have acquired the infection during the course of their investigations of undulant fever and Huddleson has reported cases of laboratory infection occurring in his coworkers.

**Infection through the Respiratory Tract**—The organism has considerable powers of resistance to drying and it retains its vitality in dust contaminated by urine for considerable periods. Dust has been held responsible for the spread of the disease in the southwestern United States and in South Africa and the French Commission in 1925 inclined to the belief that manure as well as food was a factor. However many inhalation experiments with dust contaminated with urine which contained living organisms have failed to show the infectiousness of such material even though in some instances such material when directly applied to the conjunctival or respiratory mucous membranes has produced infection. The British Commission was very rarely able to produce the disease in monkeys through the inhalation of infected dust and they did not consider it a frequent source of infection. Giordano reports that Carpenter has isolated *Brucella abortus* from tonsils and in such cases droplet infection of an individual through coughing of the patient must be considered.

**Sex**—The disease is more common in males than in females. Of 45 cases reported in New York State in 1928 two thirds were in males. Hardy, Biering, Lane, Kern and others in the United States have also found that the disease occurs predominantly in the male sex.

In Hardy's series of a total of 375 cases 289 (77 per cent) were males and 86 (23 per cent) were females. Among 186 adults living on farms 162 (87 per cent) were males and 24 (13 per cent) were females. Madsen reports that in Denmark the males outnumbered the females by 166 to 56 and Weigmann in Schleswig Holstein reported that of 27 cases occurring there 21 were in males while in Kiel he found 85 were in males and 34 in females. In all probability a proportion of the cases in farming communities are the result of direct contact with infected animals or fresh meat and in such contact the number of males obviously greatly exceeds the females. Furthermore the male population of many farms also exceeds the female. However it seems clear that males are not more susceptible than females when equally exposed to infection.

In the urban studies conducted by Hardy, Farbar and Mathews, Jones, Simpson and Giordano in which contact with live stock was practically absent the disease occurred with almost equal frequency among men and women.

In the Dayton series the females (49) outnumbered the males (41). In Hardy's group of 125 cases persons who had no direct contact with live stock or carcasses 64 (51 per cent) were males and 61 (49 per cent) were females. In Malta De Bono (1930) reports there is little difference in the two sexes as regards incidence but that the mortality is higher in females.

**Age**—All observers are agreed that the disease is much more common in middle life, rather than in early childhood or old age, the majority of the cases being among young and middle aged men. Manson Bahr gives from 6 to 30 years as the most susceptible age.

Simpson says that most children appear to possess some relative immunity to the infection as do calves. In general it is not often found in children under 12 years of age. The scarcity of the disease in children might be thought strange since they habitually use more milk than adults. It is possible of course that infection may frequently occur in the early years of life as so often happens in tuberculosis and that an immunity may develop without apparent disease or other mild infection or if an infection does occur its nature may not be recognized.

Dalrymple Champneys in his report to the British Ministry of Health on undulant fever concluded that children under 5 years are almost exempt in that country. However Gibson has recently reported a case of infection in England in a child 20 months of age. DeBono (1930) thinks the disease in Malta is common in children under 5 and may frequently be unrecognized.

Simpson found in this country 9 instances of *Brucella abortus* infection in children between the ages of 6 and 10. Kohlbrügge has recently reported the disease in a one year old infant and Hill and Monger in a child 7 months of age. Hardy in the United States in 712 cases however found only 4 under 4 years of age and a total of only 20 under 10.

Madsen in Denmark also found no cases in children under 8 and states that undulant fever has never been observed in the hospitals or asylums for children in Copenhagen where raw milk is used in large quantities.

However Larsen and Edgwick in this country examined the sera of 425 children by complement fixation tests and found that 17 per cent showed antibodies for *Brucella abortus* while 4 children who had never used cow's milk were negative. Guest (1930) tested the blood serum of 50 children in the City of Boston for *Brucella abortus* agglutinins and found only one reacting positively through dilutions of 1:40.

**Contact**—Madsen found that in Denmark in no instance was there more than one case in the same family. In Simpson's series in 8 families more than one member was affected. Hardy found that in 10 instances more than one case occurred in the same household in 3 there were 3 cases in one the entire family—father, mother and 5 children under 9 years of age—were infected.

**Occupation**—Occupation plays a definite role in etiology because of the opportunities it provides for infection.

Of 7 cases which were analyzed by Kern 6 were farmers (3 infected by aborting cows, 1 by hog carcasses), 3 were farmers' wives and 1 was a tractor manager who spent much of his time on farms. Occupations which gave contact with infected carcasses included 1 butcher, 1 meat packer and 1 laboratory technician who went frequently to an abattoir for material. Two were graduate students in bacteriology who were working with *Brucella abortus*. Both of them had been drinking raw milk. In the other 12 patients the occupations were general and non agricultural in character in which there was no special opportunity for contact infection. In Hardy's series there were farmers 62 (44.7 per cent), women on the farms 24 (6.6 per cent), stock buyers 5 (1.4 per cent), packing house employees 37 (0.2 per cent), butchers 2 (0.5 per cent), housewives other than farmers' wives 37 (10.2 per cent), students 18 (4.9 per cent), children 9 (3.3 per cent), professional and business persons and laborers 58 (16 per cent). In Simpson's series 22 were housewives, 9 were farmers or dairy men, 7 were students while the remainder were engaged in non agricultural pursuits. In Weigmann's series 10 were farmers or farm laborers, were bailiffs, a veterinary surgeon, 1 a physician, 1 a slaughterer and 12 followed other non agricultural occupations. Gentry and Fernbach in Texas found the majority of cases to occur in goat raisers and their families. Bacteriologists and laboratory workers are especially liable to infection. Dubois and Sollier in 480 cases of undulant fever occurring in France found that approximately 87 per cent of the cases occurred in those who were in intimate contact with infected animals such as shepherds, farmers and farm laborers, butchers and other slaughter house workers.

Scott (1939) give the following percentages in regard to the disease as an occupational one in different countries: Slaughterers in Great Britain 13.1, Hungary 2.6, United States 13.7, the Argentine 10.8, Veterinarians Great Britain 20.6, France 25.0, Denmark 23.4, the Argentine 26.4, the United States 12.9, Dairy and Farm employees Germany 14.1, Hungary 15.9, New Zealand 16.4, the Argentine 11.8 per cent.

On the other hand Atwood and Hissetine in Ware County Georgia found 9 cases distributed among 6 different occupations and in no case was there an occupation that

could be considered as carrying a special hazard with respect to undulant fever. All were engaged in non agricultural pursuits.

**Residence**—The incidence of the disease in small communities is usually greater. In such districts there is greater opportunity for contact with infected animals. Fewer pasteurization plants exist and raw milk is more generally consumed.

In the 35 cases of *Brucella abortus* infection occurring in the United States analyzed by Giordano and Sensenich only 6 patients lived in towns of more than 50 000 the remaining occurring in small communities of less than 20 000. Hardy in his analysis of 125 cases occurring in Iowa found 65 patients living on farms of which 11 resided in towns of less than 1000 population and 21 in towns of from 1000 to 5000 population only 23 lived in cities of more than 10 000 population and 12 of the 23 urban cases occurred among packing house workers. In Denmark Madsen found of 209 cases 145 living in rural districts and 64 in towns or cities. Atwood and Hasseltine found that 9 of the 11 cases which occurred in Georgia resided in the city of Waycross with a population of 23 000. All of these cases, however used raw milk and the cows of the dairy herds from which the milk came reacted positively to the *abortus* agglutination test.

**Seasonal Prevalence**—In the Mediterranean regions although undulant fever is present throughout the year the warm dry months from June to September give the highest incidence and December January and February the lowest. This is explained not only by the greater use of milk during the summer months but also by the fact that following the birth of the kid in the spring the contamination of the milk with *Brucella abortus* is more marked.

In Texas Gentry and Ferenbaugh found the majority of cases of undulant fever in goat ranchers from March to July a period which also embraces the kidding season in the goats and the time when the goats are in full milk. During this period all the members of the family are in direct contact with the goat herds caring for the kids and teaching them to suckle.

In the United States Hardy found that infections occurred in every month of the year apparently reaching a maximum during the summer months probably because of the prevalence of contamination of milk at that period. Hasseltine's figures for 1929 and 1930 show that the greatest number of cases occurred in each year from May to November. In 1929 the peak was reached in September but in 1930 the greatest number of cases occurred in July. However as these data cover but 2 years no definite conclusions as to normal variation can as yet be made. In the United States the consumption of milk and milk products is increased considerably during the warm months.

## PATHOLOGY

The mortality from the disease is not great and the number of autopsies with histological examinations reported has been comparatively small.

**Spleen**—The condition of the spleen is perhaps the most striking pathological condition. It is generally enlarged when death has been due to the undulant fever infection and not from some other accompanying disease or accident.

Hughes in his report of 16 fatal cases in Malta found that it varied in weight from 10 to 44 oz (283.5 to 1247.4 gm) the average being about 20 oz (567 gm). In 14 more chronic cases the average weight was but 14.4 oz (408.24 gm). In the author's 2 fatal cases in the Philippines it weighed 390 and 480 gm respectively. In Archibald's

case in the Sudan it weighed 670 gm. Bassett Smith reported a case dying after 18 months where the spleen weighed 56 oz (1587.6 gm). Löffler and von Albertini and Schöttmüller have recently reported cases of splenomegaly due to *Brucella abortus* infection. In Löffler's case there was anaemia and the spleen on account of its enlargement was removed before the diagnosis of *Brucella abortus* infection was made. In a fatal case reported by Ebskov the spleen extended nearly to the umbilical line before death. In Hab's fatal case in Germany both the spleen and liver showed a chronic inflammatory condition. Kern in an analysis of 36 cases of *B. cella abortus* infection reported by different authors in the United States notes that only 2 fatal cases are referred to in one of which (Moore and Carpenter's patient) it is merely mentioned that there was septic splenomegaly at necropsy the other findings not being given. In Scott and Saphir's case the spleen weighed 550 gm. Hardy reported 10 fatal cases. In only 2 were necropsies performed. In one of these it is merely stated that the spleen was enlarged. In the other it is noted that there was complete absence of any notable gross pathological changes in the organs of the abdominal and chest cavities. The diagnosis in this case was made only by the agglutination test.

Duffie also reported 2 fatal cases in which the diagnosis was also made by the agglutination test in low titer. In one of the cases the spleen was about twice the normal size. In the other the size of the spleen was given as normal. In De la Chapelle's case the spleen was greatly enlarged weighing 1035 gm.

Hardy later (1940) refers to 6 fatal cases. In only 3 was the condition of the spleen noted. In one case it was enlarged and in 2 no enlargement was observed. In 3 of the cases no focal necroses were found in the histological examination. However Menefee and Poston (1938) state that in their case due to *Br. suis* infection small nodular lesions were present in the lungs, liver, spleen and lymphatic glands.

The condition of the spleen at autopsy obviously varies considerably according to the stage of the disease in which death occurs. In the acute stage it is soft and diffuent but in the chronic cases it may take on the aspects of a true splenomegaly with an increase of lymphoid tissue and particularly of fibrous tissue. In Löffler's case the histological condition resembled that seen in Banti's disease. Cantani *et al.* found very large spleens simulating those found in splenic anaemia and mistaken for that condition in 6 of the cases. However in all of these the fever had lasted for 6 months or longer. Small haemorrhages and infarcts have sometimes been observed in the spleen. In the acute cases the sinuses are dilated there is active endothelial proliferation and the lymphocytes are increased.

*Histology* — In the author's fatal cases in which *Brucella melitensis* was isolated in pure culture the histological examination showed that the lumina of the capillaries and veins were markedly widened. There was engorgement of the vessels the sinuses being pressed apart and filled with red blood cells. The malpighian bodies were swollen. The small round lymphoid cells increased in number. Karyokinetic figures were frequently observed in the follicles. No abscesses or areas of focal necrosis were present. Some of the endothelial cells were swollen and granular others proliferating. The nuclei in some were multiple. In one of the cases a few microorganisms were seen scattered here and there throughout the sinuses. The most striking change was in the proliferation of the reticular endothelium of the blood and lymphatic vessels with a moderate concentration of wandering cells.

In a case examined by Lille reticulo endothelial hyperplasia of the lymph glands was noted. In Archibald's case the histological exami-

nation showed evidence of a general congestion with a hyperplasia of the lymphoid elements and marked cellular infiltration composed chiefly of large lymphocytes and large mononuclears especially around the blood vessels. *Brucella melitensis* was isolated in cultures from the spleen. In De La Chapelle's case in which death was due to vegetative endocarditis a diagnosis was also made of 'massive septic splenomegaly with multiple anaemic infarctions'. The sections of the spleen showed 'marked congestion scattered foci of necrosis, and a small area of anaemic infarction'.

Unfortunately there is no report of any cultures being made from these lesions. Menefee as noted reported small nodular lesions in the spleen and other viscera. In none of the other reported human cases that the writer knows of have necrotic focal lesions been reported in the spleen and in none have there been described lesions identical with those observed after the inoculation of guinea pigs with *Brucella abortus* or *suis* strains (The lesions in guinea pigs are discussed on p. 749). Hughes makes no mention of such a lesion in his 6 fatal cases. In the 2 fatal cases reported by Hardy, the histological examinations were made by Lille. In one it is stated that there were no focal lesions present in the spleen in the other the follicles were found to be of moderate size a few showing in the center large swollen reticular cells with cloudy appearing oxyphile cytoplasm which appeared very finely granulated. The pulp contained a considerable amount of blood a few leucocytes and macrophages and moderate numbers of lymph cells. There was reticular endothelial hyperplasia in the lymph glands.

Gregerson and Lund found a pronounced enlargement of the spleen with follicular atrophy and marked over supply of blood with eventual hyperplasia of the pulp. Parenchymatous degeneration in the organs was presumed to be due to the toxic effect of the infection.

**Lymphatic Glands**—The mesenteric glands are usually moderately enlarged and may show reticular endothelial hyperplasia. A similar condition has also been noted in the more superficial lymphatics. Schott muller suggests that the bacilli may lodge first in the mesenteric lymph nodes. By way of the thoracic duct they are then brought into the blood stream, and by way of the portal vein they also enter the liver and spleen.

**Intestines**—In the intestines there usually is no inflammation of Peyer's patches or of the solitary follicles. In a few instances patches of congestion have been noted of the mucosa of the stomach and intestine.

In the writer's cases, the histological examination of sections of the small intestines showed no ulceration and no destruction of the epithelial cells of the mucosa which was continuous over the Peyer's patches and was not thickened. Ulcerations in uncomplicated cases are probably exceedingly rare. Ivarsson noted intestinal haemorrhage in one fatal case and Bousfield found haemorrhage of the intestine in 2 and ulceration of the small intestine in one. In the few cases in which ulcerations of the small intestine have been described in earlier years the diagnosis was perhaps confused with typhoid fever or tuberculosis. In Bousfield's case at autopsy 8 definite ulcerations were found in the small intestines between 18 and 36 in from the ileocecal valve. Bousfield cut a portion of the spleen and some enlarged mesenteric lymphatic glands to Kennedy who made cultures from them. A pure culture of *Mycobacterium melitensis* was obtained from the spleen. From the mesenteric glands 6 cultures were taken 2 were sterile.

and the other 4 contained *Bacillus marsecticus* and *Bacillus coli*. No *Bacillus typhosus* was present. One colony of *Micrococcus melitensis* was obtained from the 6 cultures. Apparently there was no other bacteriological examination made and no special search made for *Bacillus typhosus*. In this case it is difficult to decide whether concomitant infection with typhoid fever or tuberculosis was not present. Regarding this point Bousfield himself says: "I believe the most skillful pathologist would have been unable to state that the ulcerations found at the postmortem were not those of typhoid fever." In more recent autopsies no mention of ulceration of the small intestine has been made. Nyquist in one fatal case reported no ulcerations of the intestine. In cases of long standing superficial secondary ulcerations of the colon have been noted in undulant fever in a few cases.

**Liver.**—The liver is sometimes enlarged and may show congestion and cloudy swelling. Microscopically there is often found round cell infiltration between the lobules and in the chronic cases evidences of fibrosis. A rare complication reported once only by Eyre and Fawcett consisted of a subdiaphragmatic and hepatic abscess. In one of Hardy's cases the liver was said to be moderately enlarged and of the nutmeg type and in Scott and Saphir's case it was slightly enlarged weighing 2150 gm. and showed cloudy swelling and passive congestion. In De La Chapelle's case it was distinctly enlarged the lower edge reaching almost to the umbilicus and weighing 2850 gm. In one case studied histologically by Lille, centroglobular necrosis and degeneration of liver cells was observed and in the second passive congestion fatty degeneration and chronic cholecystitis. Hansmann and Schenken (1932) also found a few small focal areas of necrosis in the liver in one case. Hegler has reported 2 fatal cases which died from a complicating atrophic cirrhosis of the liver. Jaundice and ascites have been noted by several observers in cases which have died late in the disease. In Ebskov's fatal case hemolytic jaundice was particularly emphasized; ascites was also present.

**Kidneys.**—The kidneys sometimes show congestion or cloudy swelling. In a few instances glomerular nephritis has been reported. In some of the fatal cases the disease has evidently been complicated by subacute or chronic nephritis. Basstrap has reported one which was complicated by acute nephritis resulting in uraemia from which the patient succumbed.

Lille has examined 2 cases histologically. In one no lesions of the glomeruli or vessels were present. In the second there was acute toxic nephrosis. However in this second case there was a large mediastinal abscess which may have been due to another microorganism than *B. celli*.

**Lungs.**—The lungs usually show hypostatic congestion at the bases and sometimes bronchitis and areas of bronchial pneumonia. In 5 of Hughes' acute cases the lungs were inolved and showed either congestion or areas of consolidation. In the cases of longer duration in only 2 were the lungs found normal. In Scott and Saphir's cases there was also pleurisy with effusion. Jenkins has recently reported a similar case. In neither instance was *B. celli* isolated from the pleura. In Gazzarini's 53 cases 3 of which were fatal all succumbed to the pulmonary complication of bronchial pneumonia. In one of Hardy's cases symptoms and signs of lung abscess developed before death and an operation for drainage was performed. There is no report of any bacteriological examination of the lung exudate. Hardy writes that both *Brucella abortus* and *Brucella suis* were isolated from the blood of this patient during life. There is no record of an autopsy in this case.

**Heart.**—The heart muscle is sometimes soft and pale in color. In a few cases evidence of myocarditis, cloudy swelling and granular or fatty degeneration have been present. In some instances the pericardial fluid has been reported as considerably increased in amount. Endocarditis has been recently noted as associated with the disease in several cases in the United States (Moore and Carpenter, Scott and Saphir, De La Chapelle and Hardy). In regard to its occurrence in France Volle in his book on undulant fever says that no cases of endocarditis have occurred. However Lagnifoul and Arnal and Sardon in earlier years have each described one case of this complication caused by *B. celli melitensis*. In the Mediterranean cases Hughes in his series of 63 necropsies found 2 cases with vegetative endocarditis. In 3 other cases



a more chronic endocarditis was present evidently of previous origin. In Scott and Saphir's case there was an old mitral stenosis and numerous soft gray friable vegetations were present on the mitral aortic valves. They concluded that there was no actual proof that the *abortus* organism was responsible for the recent endocarditis and the case was reported as one of acute and chronic endocarditis associated with *Brucella melitensis* (*abortus* bacteremia). *Brucella abortus* was cultivated from the heart's blood but no streptococci. Moore and Carpenter have also reported a fatal case of undulant fever in which there was disclosed at autopsy an old deformed aortic valve upon which a vegetative endocarditis was implanted.

Hardy reported that there was involvement of the cardiovascular system in 3 of his fatal cases. In the first of these the report of the necropsy performed by Woodward states that the heart was hypertrophied to twice its usual size and that when removing it an abscess in the anterior mediastinum was opened. It was the size of a hen's egg and contained a bloody pus. The aorta had an erosion 1 cm in diameter and the anterior cusps were entirely destroyed. There was a mass 3 cm in diameter occupying the sinus behind the valve and connecting with the abscess in the mediastinum. No bacteriological examination was recorded of the lesion. Huddleson states that *Brucella* was isolated earlier from the heart's blood in this case. In his 2 other fatal cases no autopsies were performed and the evidences of endocarditis and of other cardiac disease were merely clinical.

In De La Chapelle's case the anatomical diagnosis was (1) massive vegetation and ulcerative endocarditis of the aortic valves (2) massive septic splenomegaly with multiple anaemic infarctions (3) subacute haemorrhagic nephritis (4) chronic parenchymatous degeneration of the liver (5) massive subperitoneal haemorrhage in the right half of the abdomen, of unknown origin (6) subcuticular and subungual petechial haemorrhages of several toes of the right foot. The microscopical examination of a section stained by Gram Weigert revealed no organisms in the massive vegetation from the heart. No cultures were evidently made from the heart lesion.

Some years ago Eyre pointed out that in undulant fever some alteration appears to take place in the walls of blood vessels which makes for ease of passage of the red cells evidenced during life by ready bruising for trivial injuries and often recalled post mortem by localized extravasations of blood at various points in the subperitoneal connective tissue. He thought that possibly this was due to irritation of the vasomotor system by *Micrococcus melitensis* toxins.

**Bones and Joints.**—Effusions into the joints have also been reported in the course of the disease from several of which *Brucella* has been cultivated. Kennedy reported purulent synovitis of the costosternal and costochondral joints and in the U. S. States Edwards (1937) has noted abscesses in the bones. Recently Weil has called attention to a case with arthritis and osteitis of the right foot in which the radiograph showed bony atrophy and blurred contours of the joints between the cuneiform and the metatarsal bones. The swelling of the dorsum of the foot gradually subsided. Simpson and Bowers in a study of 76 cases in Ohio found joint symptoms a prominent feature in about one half of the cases. Hardy and his associates found that while tenderness and pain on active motion were frequent hydrarthrosis or swelling of the joints was unusual and occurred in less than 2 per cent of their cases.

Cellulitis and myositis have been reported. The lesions are evidently not inflammatory but Paviot and his associates believe that the effusion of plasma sometimes observed accounts for the pains in the muscles so frequently complained of in this disease and especially in the muscle sheaths close to the joints.

**Generative Organs.**—The generative organs are not infrequently involved. In the male orchitis epididymitis prostatitis and seminal vesiculitis may occur and in the female catarrhal vaginitis mastitis and ovaritis. Abortion in human cases has been much rarer than in cows. Indeed in a study of the Mediterranean cases Eyre remarks that although pregnancy frequently synchronizes an attack of Malta fever its course is unaffected although lactation is frequently curtailed. Hardy observed but one infection during pregnancy. In this the condition proceeded normally. Corneli and D. Young in an attempt to study the incidence of undulant fever in pregnancy and abortion found that the blood serum from 22 or 23 women examined who had aborted

gave a negative agglutination reaction. In the 23rd case the aborted blood gave a positive reaction in a dilution of 1:80 but the venous blood was negative and cultures from the placenta were negative. One woman who had clinical symptoms of undulant fever in which the diagnosis was confirmed by agglutination tests gave birth to a normal child at term before the onset of symptoms of undulant fever.

Williams and Kolmer also examined the sera of 50 women who had aborted. Complement fixation tests in which an *abortus* antigen was employed were not more frequently positive than the Wassermann reaction. Agglutination tests with 12 sera were negative. However in other cases in the United States Simpson and Frazer found that the blood sera of 5 women who had aborted repeatedly were found to agglutinate *Brucella abortus* and *Brucella melitensis* in high titers. All of them were raw milk consumers. Some similar evidence has been reported from both Germany and Switzerland. Moreover Carpenter has isolated *Brucella abortus* from a human foetus which was aborted at the end of the fourth month of gestation. Kristensen also isolated *Brucella abortus* from the placenta of one case. It has been suggested that abortion may be more common in those cases in which the invading organism has been *Brucella abortus* rather than *Brucella melitensis*.



FIG 185.—Photograph of a placenta showing the position of the fetal surface. The fetal surface is on the left and the maternal surface is on the right. The placenta is shown in a cross-section view.

Carson has reported a fatal case in which the right uterine tube was much enlarged in diameter and the lumen contained 2 cc of sero-purulent fluid. There was a walled-off abscess area containing pus under the right broad ligament. A histological examination of tissue from the wall of the abscess showed typical abscess formation. However no cultures were apparently made from the abscess.

**Nervous System.**—Lyre found that the cerebrospinal fluid was often increased in quantity in cases which had exhibited meningeal symptoms in some instances to such an extent that there was flattening of the cortical convolutions. In other cases the brain and cerebrospinal fluid appeared normal. Kennedy also noted flattening of the cortical convolutions from the increase in the cerebrospinal fluid. Hughes in his series of necropsies in which the brain was examined in 10 found congestion of the meninges, superficial veins and choroid plexus in 9. In a few cases there was effusion into the ventricular spaces. In the early acute cases the congestion was more intense.

A case of meningitis cephalitis was observed at the Mayo Clinic (1932) from which through guinea pig inoculation *Brucella* was isolated. Sanders (1931) reported a case of meningitis which resulted fatally in which *Brucella* was isolated from the turbid spinal fluid. At necropsy when the meninges were exposed numerous grayish white tubercles were noted in the leptomeninges overlying both cerebral hemispheres. On removal of the brain a large blood clot involved the base and covered the



FIG 186 —Photomicrograph of meningeal vessel showing dense collection of lymphocytes  $\times 200$  (After Hansmann & Schenken Courtesy Am J Pathology)



FIG 187 —Photomicrograph of a small brain stem artery. The increased adventitial connective tissue is heavily infiltrated with inflammatory cells and there is marked destruction of the media with connective tissue replacement. Definite endarteritis is present  $\times 200$  (After Hansmann & Schenken Courtesy Am J Pathology)

medulla pons cerebral peduncles and the optic chiasma. After fixation a mycotic aneurism which had ruptured was discernable. *Brucella* was isolated from the tubercles, blood clot and lymph nodes but the culture attempted from the heart's blood showed no growth. Hansmann and Schenken (1932) have further reported upon this case with the addition of the histological examination (Figs 86-187). In the brain both the pia and arachnoid revealed various degrees of thickening due particularly to an inflammatory cell infiltration and connective tissue proliferation. The inflammatory cells were largely lymphocytes and plasma cells with some large mononuclear cells and a few polymorphonuclear leucocytes. Serial sections of the meningeal tubercles showed they were composed of irregular masses of hyalinized connective tissue moderately infiltrated with chronic inflammatory cells. In one area where the inflammatory cell infiltration was especially marked necrotic tissue was present in which polymorphonuclear leucocytes were noted. In another similar area the central portion was composed of large mononuclear cells surrounded by a dense collar of lymphocytes. Newly formed vessels were present in many of these inflammatory cell collections. It appeared that these areas represented various stages in the formation of a tubercle from necrosis to connective tissue hyalinization.

Rogers (1932) has analyzed the literature especially of the cases reported abroad and DeNunzio in Malta who has studied the pathological histology of the nervous system believes that the *Brucella* toxin gives rise to degenerative changes in the nerve cells with breaking up of the nerve fibrils and leucocytic infiltration most marked in the cerebrum and medulla. He also found the peripheral nerves similarly involved a condition which would explain the frequent peripheral neuritis encountered.

**Occurrence of the Organism**—At autopsy *Brucella* has been cultivated from the spleen, heart's blood, pericardial fluid and the mesenteric glands more rarely from the liver, gall bladder, kidney, the urine, supra-renal bodies, pancreas, thymus and other lymphatic glands. Cultures from the saliva and sweat have resulted negatively. There are several clinical reports of the isolation of the organism in non-fatal cases from the sputum and one from the cerebrospinal fluid and meninges and once from the pleural fluid. In several instances it has been cultivated from the effusion of the joints though often these are sterile. Wainwright and Kristensen have reported the recovery of *Brucella melitensis* and *Brucella abortus* respectively in pure culture from 2 cases of ovarian cysts and Amoss (1936) in one. The organism has also been isolated in a few cases from the faeces.

*Brucella* gives rise to general and local symptoms of a septicæmic nature whose severity is greatly dependent upon the virulence of the infective strain and its toxin. The toxin is apparently of the nature of an endotoxin and experiments upon animals have demonstrated that killed cultures may produce a similar effect to the living ones. The effect of the toxin is particularly shown in the persistent fever, irregular cardiac action and frequent palpitation and in the irritative symptoms in the nervous system. Besides these symptoms of general infection the organism may also occasionally cause local disturbances such as cold abscesses, costal swellings, osteitis and more rarely phlebitis and perhaps endocarditis. Lesions having the characteristics of pyogenic abscesses which may occasionally occur during the course of the disease are usually due to secondary infections with some other microorganism.



FIG 186 —Photomicrograph of meningeal vessel showing dense collar of lymphocytes  
 X 200 (After Hansmann & Schenken Courtesy Am J Pathology)



FIG 187 —Photomicrograph of a small brain stem artery. The necrotic distal connective tissue is heavily infiltrated with inflammatory cells and there is marked destruction of the media with connective tissue replacement. Definite endarteritis is present. X 200 (After Hansmann & Schenken Courtesy Am J Pathology)

Gwatkin has examined 5 samples of faeces from infected and 4 from uninfected cows for the presence of *B. cella abortus* bacteriophage. He also examined 9 samples of milk from infected and 4 from uninfected animals, infected and uninfected foetuses, normal foetal membranes, and 3 samples of blood from infected cattle. However, there was no evidence of the presence of bacteriophage in any of the material examined, although agglutination was produced by the filtrates of blood and by some of the milk samples from the infected cows.

### SYMPTOMATOLOGY

**Incubation Period**—The incubation period has varied usually from 5 to 17 days. Bruce from clinical experience gave it as from 6 to 17 days. Bassett Smith from 6 to 20 days, but believed that usually it is about 14 days. Hughes regarded it as 10 to 15 days as a rule, but thinks it may be considerably shorter in some cases.

In the experimental infections and those occurring in laboratories already mentioned it varied from 5 to 17 days. In 6 cases of experimental infection through the abraded skin performed by Otero in Puerto Rico, the incubation periods were from 10 to 16 days. In 2 cases in which *Brucella sm.* was fed in cultures to individuals, the incubation periods were indefinite on account of the manner in which the experiments were performed; the data suggest that they may have been 10 and 17 days. In other infections occurring through the skin and conjunctiva, the incubation period has been in some instances not more than 5 to 11 days. Subcutaneous inoculation of monkeys shows an incubation period when virulent cultures are used of about 5 days, whereas if the organ *sm.* is given by mouth the incubation period appears to be lengthened to about 15 days. Simpson says that while it is a difficult matter to determine it with accuracy, it has been found to vary in his cases from 5 to 14 days. Rainsford (1935) in Malta, who was able to measure the incubation period in 5 cases, 3 with definite evidence found in these 3 that the incubation was 20, 39 and 47 days.

**General Course. Mode of Onset**—There is nothing characteristic about the prodromal symptoms. The onset as a rule is insidious. There may be a period of weakness, general malaise, headache, pains in the back of the neck, and general muscular pain and anorexia before the appearance of the fever. Occasionally gastric disturbances and sore throat occur at the onset. As a rule, in the beginning the temperature rises gradually—in the evening to 103–104 F—as in typhoid fever, with morning remissions. Sweating and chilly sensations may be noted. In 6 of Kern's cases the onset was with chill, and in 4 of Simpson's cases also the disease was initiated with a sharp chill and a more rapid elevation of temperature to 103–105 F.

**Further Course**—Later the tongue usually becomes coated and the pharynx may be congested. Epigastric tenderness and signs of gastric catarrh may become more marked, and evidences of pulmonary congestion and bronchitis may appear. As the disease progresses the headache is often severe, and there is sleeplessness and marked irritability. The gradual rise in the temperature at the onset and the other symptoms frequently suggest typhoid fever. However, there usually are no rose spots, and constipation is generally a striking feature. Fever is the most important clinical manifestation, but it may be exceedingly variable in character. Striking features are its extreme irregularity and a great tendency to relapses, and the period which it lasts, which may be for one

## IMMUNITY

Production of immunity to *Brucella* infection seems to be exceedingly difficult. No satisfactory immunity has been produced in guinea pigs either by feeding or by the subcutaneous inoculation of cultures of *Brucella*. Some of the recent experiments in goats seem to show that these animals may sometimes though not always, be immunized by very large doses of cultures. It has become rather generally accepted that killed cultures of *Brucella abortus* have failed to demonstrate their value in the control of contagious abortion of cattle. The advantages of living cultures in this respect also have not been conclusively demonstrated. Moreover, the use of living cultures is not without danger, since Theobald Smith pointed out that the vaccinal strains may enter the udder and continue to multiply in the ducts. Many attempts have been made to produce immunity in man by killed cultures of *Brucella*. These also have not been entirely convincing of the idea that a satisfactory protective immunity can be produced. The results in both man and animals are discussed more fully in this article under the subjects of vaccine treatment and prophylactic inoculation. The presence of *Brucella* agglutinins in the blood which occurs during the course of the disease in man or following the inoculations of killed cultures in man or animals does not in itself indicate either an antitoxic or bactericidal immunity for *Brucella*. The experiments of both Durham and Eyre substantiate this fact. Eyre further showed that the blood of infected animals frequently had a high agglutinating power for some time prior to their death. On the other hand Manson Bahr points out that some of his most severe cases never had an agglutinating titer of more than 1:80. Bruce considered that one attack of undulant fever conferred immunity against subsequent ones. However Hughes observed second attacks in some individuals and Bassett Smith concluded that immunity following the disease was only slight and that secondary infections do occur. In many instances young children appear to be relatively immune to infection or if infected may acquire an immunity sometimes as in tuberculosis without visible evidences of disease.

Cotton states that calves up to the age of 3 and possibly 6 months are immune to infection although specific agglutinins may be present in the blood. However Simpson reports that in serological studies of 103 cattle 76 of the cows and all 6 of the bulls gave positive agglutinating reactions while the serum of 8 calves gave entirely negative results. It has been generally noted that *Bacillus abortus* infection rarely if ever exists in sexually immature animals and that calves seem to be wholly immune to the infection. It has been suggested that it is possible that the same situation attains during the preadolescent period in human beings.

The results of agglutination tests on individuals with no history of the disease especially veterinarians and butchers suggest that an immunity has been acquired from subclinical or unrecognized infections. The incidence of *Br. abortus* infections is low in proportion to its frequency in cattle and many of the cases are very mild. The caprine and porcine types are more pathogenic for man.

The question of a passive absorption of *abortus* agglutinins occurring in man and appearing in the blood serum after drinking infected milk is discussed further under Sources of error and Agglutination p 788

toms into septic arthritic neuralgic visceral and glandular types Eyre grouped the cases arbitrarily under the headings of (1) acute (2) subacute and (3) chronic

In the *acute or malignant type* the onset is usually sudden with rigors accompanied by temperature of 100 to 106 F severe headache often limited to the back of the eyeball indefinite pains about the trunk and limbs particularly in the back and general malaise In such cases the face is flushed the dorsum of the tongue thickly coated with white fur but pink and moist at the sides and tip or more rarely dry brown glazed and cracked and the breath offensive Diarrhoea is often present during the first few days of the attack but soon gives place to constipation The pulse is strong and increased in frequency though not usually in proportion to the temperature The urine is diminished in amount high in color and contains large quantities of uric acid and urates This type of fever

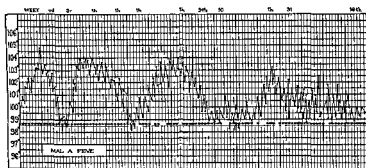


FIG 88—T m p t u h t f u d l n t f e v e (Aft S h u b )

sometimes passes into the typhoid state and death results from cardiac failure or more rarely hyperpyrexia supervenes Sometimes a crisis occurs and recovery takes place but usually the temperature gradually falls to or near normal and the case assumes the subacute type

The *subacute type* corresponding to the *undulant* one of Hughes is often slow and gradual in onset For some days slight headache thirst constipation and gastric disturbances pains in the back neck and limbs (usually described as rheumatic) accompanied by insomnia mental anxiety and general depression combine to produce a marked but at the same time indefinite feeling of ill health Next there follows a steady and gradually increasing rise of evening temperature until 103.5 to 106 F is reached with morning remissions followed by a similar and almost equally gradual fall until the morning temperature becomes practically normal The remissions of temperature are almost invariably accompanied by profuse perspiration The duration of the initial pyrexial attack varies in different cases from 1 to 5 weeks Then after an apyrexial interval lasting from 5 to 10 days or a fortnight during which the temperature remains at or about normal a relapse sets in similar in all respects

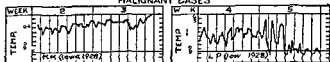


or several weeks a few months or even years Chilly sensations and profuse sweating are frequent symptoms As the disease progresses the spleen becomes enlarged and frequently palpable below the costal margin and in some instances late in the disease the liver may also show enlargement Pain in the joints and abdominal or lumbar pain may be severe Rheumatic like pains in the joints and fasciae have been noted roughly in from one third to one half the cases Orchitis mammitis and neuritis may also occur In about three fourths of the cases cranial or facial neuralgia lumbago and sciatica or other symptoms of neuritis rarely with slight paralysis, may appear If the fever continues there may be marked evidences of a septicaemia and other irritative evidences of the toxin upon the nervous system The patient is often restless at night and cannot sleep Nervous prostration is sometimes marked with muttering delirium and there may be involuntary passage of urine and drowsiness passing into stupor In severe cases the sweats are generally profuse and distressing particularly at night A secondary anaemia may occur with a loss of 20 to 50 per cent in the red blood cells and with an equal or even greater reduction in the haemoglobin In severe cases the pulse often becomes rapid, and murmurs may be heard In malignant cases death may occur in from 5 to 21 days from hyperpyrexia or cardiac disturbances or complications of the lung Loss of weight is usual in most of the cases and in the advanced stages many of the patients become considerably emaciated Loss of weight has been especially emphasized by both Hardy and Simpson in the American cases In only 10 per cent of Hardy's cases was there no apparent loss of weight in Simpson's series it was almost constant One patient lost 62 lbs in a period of 6 weeks and 4 lost more than 50 lbs 34 lost between 24 and 50 lbs, while there were only 10 patients who experienced no appreciable loss of weight

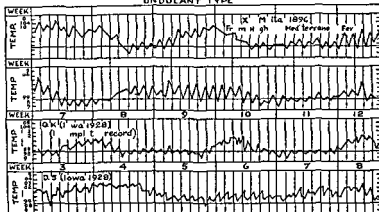
**Differentiation of Types of the Disease**—All clinicians who have extensively observed the disease emphasize the extreme variability of the symptoms At times it may simulate typhoid fever acute rheumatism tuberculosis or septicaemia due to other microorganisms Hughes says so variable are the symptoms and so uncertain is the duration and course of this fever that it is impossible to give a description to which all cases can be referred For this reason he divided the disease into 3 clinical types (1) malignant (2) undulant and (3) intermittent He regarded the undulant type of fever as the usual one the other types being but variations brought about by differences in severity The febrile course in this type was marked by intermittent waves or undulations of more or less remittent pyrexia of variable length separated from one another by periods of temporary abatement or absence of symptoms In addition to these types however he described irregular and mixed types

Thomaselli enumerated 4 clinical types (1) the gastric (2) the indeterminate (3) the nervous and (4) the lethal or paralytic while Bassett Smith distinguished 5 types (1) ambulant (2) mild (3) the most common form, (4) malignant and (5) intermittent Giordano and Sensenich divided their cases in the United States on the basis of predominant symp

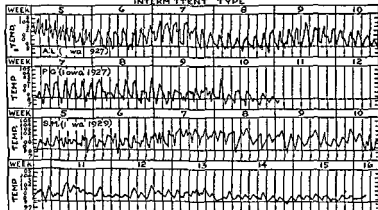
## MALIGNANT CASES



## UNDULANT TYPE



## INTERMITTENT TYPE



## ABRUPT CASE

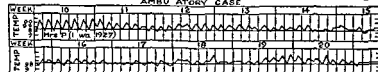


FIG 189.—Temperature undulant types of different low with  
 n h r t f m Hugh (After H dy C urt y U S Public He lth Service)

to the first attack, but often distinctly shorter and less severe. This sequence of events is repeated again and again, the duration of the disease varying from 6 weeks to 6 or 9 months. In rarer instances the disease may continue for 2 years and more rarely with typical pyrexial attacks at irregular intervals for 3 years.

In the United States about 15 per cent of the cases have been of the undulant type. Stitt, in contrasting the infection of caprine origin with the cases of porcine and bovine source as seen in the United States notes the following outstanding features in the latter group: remarkable absence of physical signs (including infrequent splenomegaly); profuse redolent sweating; loss of body weight, fatigability with absence of prostration; joint manifestations and neuralgias not so common or severe; abdominal pains more prominent even leading to unnecessary operation; orchitis less commonly; prostatitis, and vesiculitis.

In the ambulatory type first referred to by Shaw, the symptoms are said to be entirely absent, or are limited to a few days of low fever. The only proof of the existence of the infection may be the presence of agglutinins in the blood and occasionally of *Brucella melitensis* itself there while in the urine although normal in appearance, the specific organism has sometimes been reported in enormous numbers (22 000 per cc) and in a highly virulent condition. In other cases the fever may last for only about 6 days but the weakness and irritability may endure for weeks. In some of these cases the diagnosis has been made by blood culture. Some of these cases are probably regarded as attacks of influenza.

In Hughes and Bassett Smith's intermediate type the particular features are a more or less daily intermitting temperature, the fever being less intense but more hectic in character than in the other types. The onset is generally insidious, the general course milder and relapses are less frequent but constipation, sweats, joint pains and neuritis may be present. However in such cases at any time the fever may change to that seen in the undulatory or malignant types.

On account of the failure of many cases of the disease to conform to any one type perhaps a consideration of the more prominent symptoms as they occur in all these different types may give a more concise idea of the clinical picture of the disease rather than an attempt to classify them into rigid types.

**Detailed Consideration of Special Symptoms.** *Fever*.—The elevation of temperature is clearly the chief and sometimes the only clinical manifestation of undulant fever. The pyrexia is characterized particularly by its variability in degree and duration and in the tendency of the daily maximum and minimum temperatures to form more or less definite waves or undulations of varying character and length. These waves of temperature however throughout the course of the disease may never be very marked or striking. There may be only one wave or there may be a secondary period of fever which might be referred to as in the nature of a relapse. The periods of fever are not sharply marked as they often are in African tick or European relapsing fever for example and it is only in rather exceptional instances that the clinician may be able to recognize repeated undulations in the temperature throughout its course.

In earlier years owing to a lack of knowledge of precise laboratory methods for the diagnosis of infectious disease there was a greater tendency for the physician to lay

that the percentage of recurrences may be increased as these cases are studied over a longer period. In Kern's collected cases undulations of fever were present in 9 of the 21 in which the curve was given. Hardy observed very few temperature curves of the malignant type and only a small percentage of these had definite undulations. However delirium occurred in 6 of Simpson's cases in which the fever reached great heights—106 to 107 F. Hardy found the intermittent type not uncommon and followed by one or two relapses usually of short duration which came after a few days or even after a period of months of apyrexia. The usual chart showed an intermittent fever, the temperature gradually increasing during the period of invasion and disappearing by a slow lysis. In ambulatory cases the height of the temperature was variable and was readily increased by overexertion. Both Hardy and Simpson found that the fever was often a great disparity between the accepted sense of feverishness and the extent of the fever as registered by the clinical thermometer. In many instances the patient neither presented a febrile appearance nor had he complained of feverishness, but the physician found to his great surprise a temperature of 102 to 103 F. Hughes also called attention to this condition in a study of the Mediterranean cases.

Chills and sweats are striking symptoms in many cases and are obviously connected with the fever and influence its fluctuations. Sweating was so characteristic a feature in the Mediterranean cases that in Italy the disease was described by Tomaselli as *febris sudoralis* on account of this symptom. Hughes compared it with the night sweats of phthisis and those accompanying the hectic fever of other suppurating conditions but remarked that it was much more profuse. The condition is most debilitating and depressing and seems to increase in severity as the disease progresses and the patient becomes weaker. These profuse perspirations follow the diurnal fall of temperature their time of onset being governed therefore by the pyrexial curve of each individual case. In the greater number of instances the sweating occurs between 11 P.M. and 2 or 3 the next morning. Simpson also found that the perspiration usually occurred during the early morning hours in about one half of his cases and was of a drenching character. He remarked that in 2 such patients the sweats were the most impressive feature of the disease since the sense of weakness is pronounced during and immediately after this experience. Hardy also noted sweating as the most distinctive feature of the disease which occurred in 84 per cent of his cases. In 53 per cent the sweating was profuse or moderately so. It usually occurred soon after midnight and was of short duration. In a smaller number of instances however it was quite prolonged necessitating several changes of linen during a single night.

Chilliness or true chills in the period of invasion of the disease has already been referred to. However during the course of the disease chilliness and true rigors also occur. In 14 of Simpson's cases the chills usually one day were of sufficient severity to be regarded as true rigors. However in 8 of his patients who experienced fever and sweats chills were absent. True chills were also a feature of more than one third of Hardy's cases though in but 12 per cent did more than two occur. When they appeared early they frequently led to a diagnosis of pneumonia and when they developed during the course of the disease and recurred regularly they suggested malaria. In an occasion like this there was more than one in 4 hours one patient reported a day for several days in succession and another stated that on one day he had 5. In his mild infections rigors were not noted but in the severe cases they were common. In Kern's cases drenching sweats were reported in 1 and faint chills in 3.

**Variations in Fever.**—Orpen in his discussion of 35 cases of *abortus* infection in South Africa expresses the opinion that the fever curves are more variable than in true Malta fever. Viviani found the fever in his Italian patients to be rather of the continued type more often than is usually described in the Mediterranean cases and Fiscali and Alessandrini likewise reported fewer undulations of temperature in their cases suspected to be of bovine origin. Giordano and Sensevich emphasize in their cases in the United States the relative infrequency of the classical undulant

stress upon the type of fever for diagnosis and indeed sometimes to attempt to diagnose the infection by the type of fever. More or less rigid descriptions of febrile conditions thus found their way into the literature.

In the classical undulating type of Hughes the temperature usually rises gradually through a week to 10 days to between 103 to 104 F. It is usually of an intermittent character rising towards the early afternoon or evening from 2 to 4 degrees reaching its maximum usually by 6 p.m. and falling during the night the patient sweating profusely in the early hours of the morning. After 1 to several weeks of such fever the temperature begins to abate gradually and mild cases may reach normal in 2 or 3 weeks. This may end the attack. In the majority of cases however after a few days of relative or absolute apyrexia have occurred the temperature begins to rise again gradually reaching 103 to 105 F. and the patient suffers from a second attack of fever resembling the first. Other attacks or relapses of fever may follow through several months. There is usually a tendency for the relapses to decrease in length and severity as the disease progresses. The waves of fever may average from 7 to 10 days in length or more rarely they may be as long as about 3 weeks or even longer. Hughes reported the average number of relapses was 3 or 4 but as many as 6 or 7 frequently occurred. Intervals of apyrexia between the relapses were usually from 3 to 5 days but might vary from 1 to 10 days. In Hughes series of cases of this type the duration of the fever varied from 20 to 300 days the average duration being 60 days and the average stay in the hospital 90 days.

In the intermittent type the fever also often begins insidiously assuming an intermittent character with distinct daily intermissions and the diurnal range may be as much as 3 to 4 degrees. However the fever in this type is usually less intensive and may only range from 99 or 100 F. in the morning to 101 to 103 F. in the afternoon. Sometimes the temperature suggests a septic condition and may continue for several months without further symptoms except slight sweats constipation increasing debility and anaemia. The general course of the disease however is usually milder than in the undulant type and the relapses are less frequent.

In the ambulatory type the fever is often even more benign and irregular than in the intermittent one and the patient may either show very slight or no symptoms or may complain merely of some weakness and of being feverish. San Roman found this ambulatory type to be very common in the outbreak which occurred in Spain in 1914.

In the malignant type the temperature often rises more suddenly at the onset and the fever becomes of the high continued type reaching 104 or 105 or occasionally 106 F. or even 108 shortly before death. Hyperpyrexia although rare is a serious and often fatal complication. While it may appear at any time during the course of the disease it usually follows a continuously high temperature either early in the malignant cases or during a severe relapse.

Eyre in an analysis of 1000 temperature curves of European cases found 58 per cent with remittent 26 per cent with continuous 14 per cent with intermittent fever and hyperpyrexia in 2 per cent.

However it should again be emphasized that the majority of cases of undulant fever cannot be classified as conforming to any single one of these types. What is more often seen is the combination of them or successive transformations from one type to the other. In many cases the irregularity of the temperature chart partially or completely hides the succession of the relapses or waves of fever.

Thus Hardy found that in a study of the temperature charts of the cases he analyzed in the United States he did not encounter a single chart which conformed closely to the undulant fever type so frequently described as characteristic of the Mediterranean cases. A few of Hardy's cases showed definite undulations with periods of apyrexia although all had a rather low grade fever. Although complete temperature records were not available in these cases he thought that undulatory pyrexial relapses occurred in less than 15 per cent and in these this feature was rarely outstanding. Simpson Bierring Sensinich and Giordano also found recurring febrile relapses the exception rather than the rule. Recurring undulations of fever appeared in only 11 of Simpson's 90 cases. In 89 per cent the patients experienced but one febrile period lasting from one week to several months and finally reaching the normal level by lysis. He points out however

insufficiency Pericarditis has been reported in a fatal case and mycotic aneurysm of the basal artery in two cases

*Phlebitis* has been observed in some instances Ivarson has also reported thrombosis of the femoral vein in a fatal case Violle states that phlebitis as a complication is not very uncommon in France He however gives no details of any specific cases Hegler also has reported thrombosis of the veins in 2 fatal cases However in neither of these was *Brucella* isolated at autopsy

**Haemorrhages**—Epistaxis is not common at the onset of the disease but not infrequently occurs during its course and in severe cases haemorrhages from the gums intestine and even the stomach have sometimes been reported

Passett Smith has referred to a fatal case in which the haemorrhage from mucous surfaces hastened death Castarini reported marked haemorrhagic manifestations in a boy of 3½ years of age Profuse epistaxis occurred in the second week Following this punctiform subcutaneous haemorrhagic spots of variable size appeared over the entire cutaneous surface but especially on the lower limbs With the subsidence of the fever the lesions disappeared However 40 days later a relapse occurred and haemorrhagic spots were again present with transitory oedema of the eyelids hands and feet The haemorrhagic manifestations later disappeared with the fever and the patient recovered Bassett Smith Manson Bahr and Castronovo have also noted purpuric haemorrhagic lesions Poddighe has particularly emphasized haemorrhagic manifestations in an epidemic of the disease in Sardinia in which the clinical picture was that of an extremely grave haemorrhagic septicaemia with haemorrhages from the kidney lung and serous membranes Ivarson has reported 1 and Bousefield 2 cases with intestinal haemorrhage all terminated fatally

The blood pressure in those instances in which it has been taken has been usually below normal Hardy observed that a low blood pressure though rarely of marked degree may occur late in the disease

**Blood**—The blood has been studied in European cases by Bassett Smith and by Rainsford (1935) and in the United States recently by Simpson and Hardy Kern Munger (1939) and others Similar changes have been noted by all of these observers A secondary anaemia is usually present varying in amount according to the severity and duration of the disease The red blood corpuscles become gradually reduced in number as the disease progresses together with a loss of the haemoglobin In a few instances the red blood count has been normal The lowest recorded count by Bassett Smith was 2 500 000 and by Kern 2 800 000 Nucleated red cells are rarely seen Bassett Smith found in some cases microcytes and macrocytes abundant and metachromatism or minute basic stippling of the cells as seen in lead poisoning malarial cachexia and several other conditions The white cells are also usually decreased in number and the polymorphonuclear cells relatively decreased while the lymphocytes are increased However the leukopenia is often not marked

Simpson gives the count of white cells in 90 cases as a rule of 4000 to 6000 with a lymphocytosis having occurred in all but 12 of his cases In 8 the lymphocytosis exceeded 50 per cent In only 2 cases did the leucocyte count exceed 10 000 and in the remaining 6 cases it was within normal limits Hardy also found that the differential count usually revealed a lymphocytosis with large mononuclear cells predominating and some pathological forms while the eosinophils and basophils did not show any essential change from the normal A leucocytosis is uncommon but has been recorded in a few instances particularly when complications have supervened

type of temperature so often described in *Brucella melitensis* infection Hardy also says the one feature which overshadows all others in the description of undulant fever of caprine origin is the undulatory type of temperature a rare finding in that of bovine or porcine origin He further suggests that the rigors are a more striking feature in the cases of bovine or porcine origin than in those of caprine origin He emphasizes that the fever of bovine or porcine origin is most commonly of the intermittent type in contrast to that of caprine origin, where the undulatory type predominates

**Circulatory System**—Cardiac irregularity and palpitation from slight exertion and precordial pain are often present during the course of the disease Such an irritability of the heart has been frequently referred to in the reports of the European cases and has been attributed either to implication of the vasomotor nervous system or to direct irritation of the cardiac muscle by the toxins of *Brucella* present in the circulatory blood

Hardy in the American cases also noted palpitation and the symptoms of an irritable heart occurring during the course of the disease Dizziness was at times complained of either early in the course or during the height of the disease Blurring of vision was a symptom noted by Giordano and Ableson in 5 of their patients with no objective physical signs to account for it

In only exceptional cases has a diagnosis of myocarditis been made In general the pulse is usually elevated in proportion to the temperature The pulse is occasionally rapid but other patients showed a slower pulse similar to that of typhoid Kern notes one case in which tachycardia was present but no case with bradycardia Simpson on the other hand says that in 60 per cent of his cases the pulse rate was disproportionately slow during the febrile periods In the remaining minority the elevation of fever was paralleled by a proportionate increase in pulse rate Cardiovascular symptoms were not regarded as of importance in most of his cases Haemic murmurs have been met with particularly in anaemic and debilitated subjects and in association with cardiac complications

**Endocarditis** as a complication has been referred to in the discussion of the morbid anatomy Hardy has reported symptoms and signs of malignant endocarditis in 1 of his fatal cases, in which however there were no necroses

In another case a patient with a past rheumatic history and a well-compensated mitral lesion developed an auricular fibrillation early in the attack Throughout the illness cardiac symptoms were prominent and following the subsidence of the fever he failed to gain and died a few months later De La Chapelle has also reported one case of fatal endocarditis which he believed was due to *Brucella melitensis* Bassett Smith found that it occurred twice in a series of 750 cases but the details are not given

**Pericardial effusions** may also occur In 2 of Hughes cases in which the effusions were extensive death occurred one on the nineteenth and the other on the sixty second day of the infection and vegetations upon the mitral valve were present in both

In De La Chapelle's case roentgen ray examination showed enlargement of the heart and at necropsy the pericardial cavity contained fully 500 cc of fluid In Ebskov's case the patient suffered from palpitations dyspnoea and oppression and at the necropsy there was extensive hydropericardium hypertrophy of the left ventricle and aortic

was also cultivated from the spleen. Carpenter and Boak and Chapman have recorded 3 instances with negative agglutination in which they isolated *Brucella abortus* and Evans (1939) has reported four such cases.

Taylor and his associates (1938) in France succeeded in isolating *Brucellae* from 39 specimens of blood which failed to produce agglutination or agglutinated in titers of less than 1:80 and of these 39 specimens 27 were collected 30 days or more after the onset of the disease. The inter-agglutinability between strains of *Brucella Pasteurella* and *Pfeifferella* by specific serums is still a debatable question among many investigators. Mallmann and others have observed a high degree of inter-agglutinability between strains of these groups.

**Blood Culture**—During the early and acute stages of the disease the organism can frequently be isolated from the blood by culture. In 60 cases in which blood culture was made in the United States in 10 (50 per cent) one or more positive cultures were obtained. In 10 patients in which from one to 4 cultures were made all were sterile. In one of Simpson's cases the organism was finally recovered from the blood after 6 negative results. In 10 of his patients admitted to the hospital the organism was isolated from the blood in 7.

Huddleson (1937) made cultures on bacterio-tryptose broth from the blood of 55 cases of brucellosis in Malta. Of these cases 38 were febrile and 7 afebrile at the time he made the culture. Positive cultures were obtained in 32 cases of the former group and in 5 of the latter.

Taylor (1938) in France obtained positive blood cultures in 55 per cent of the cases which gave an agglutination reaction in 1:80 or above. The percentage of positive cultivations increased with the agglutination titer of the blood until it reached 1:64. After this dilution of agglutination there was no significant difference. In other series of cases the organism has been isolated from the blood by culture in from 54 to 82 per cent.

**Respiratory Symptoms**—These vary greatly according to the severity and length of the disease. Catarrhal bronchitis has been frequently encountered in the later stages and broncho-pneumonia is not uncommon. The 3 fatal cases of 53 reported by Gozzarini succumbed to broncho-pneumonia. In cases of long standing there is always more or less hypostatic congestion and in the rapidly fatal or malignant type hypostatic pneumonia may occur. Hughes found evidence of basal congestion in 95 per cent of his protracted cases. Hardy noted in a series of 175 that more than one third of the patients had a cough, some with mucoid or mucopurulent sputum while 10 per cent of his cases had moist and dry rales indicative of bronchitis. In 2 of the severe infections the diagnosis was broncho-pneumonia. In one a pulmonary abscess developed at the end of an infection in which the respiratory symptoms had been prominent throughout. It is not clear that the abscess was related to the *Brucella* infection. In the series of 26 cases which Kern analyzed pulmonary symptoms were markedly absent or unrecorded. A few rales were noted in only one case and in another a roentgen ray picture of the chest showed only peribronchial thickening. In the cases studied by Basset Smith and Hughes pleurisy either dry or with effusion was found to have occurred rarely. However Halbron, Pisani, Bancilhon and Poddighe in Europe



Rainsford (1935) in a study of the disease in Malta, in general confirms these observations. He emphasizes the value of leukopenia as an aid to a differential diagnosis. This in itself will distinguish it from many other infections. In his blood counts the leucocytes varied usually between 6000 and 10 000. He found that a rise in the total white cell count and in that of the mononuclears was usually accompanied by an improvement in the condition of the patient even in cases where the leukopenia persisted. As the cases improved the mononuclear count increases.

Munger (1939) in the study of 32 cases of *melitensis* *Brucellosis* noted that many of the mature small lymphocytes are larger than normal varying from 12-14  $\mu$  in diameter and he has termed them pathologic lymphocytes. As many as 30-80 per cent of the lymphocytes appeared to be of this type and they were found in 40 per cent of the patients.

Sabin (1934) by supravital staining found in cases of brucellosis an increase in a type of monocyte which is similar morphologically to one which has been associated with various forms of hepatic involvement and described in catarrhal jaundice. Isaacs has also observed this cell which he called the liver damage cell in the blood of all the cases of brucellosis he examined.

Huddleson (1940) has noted that in patients infected with *Brucella melitensis* during the period of fever there may be a marked basophilia of the granules of the nucleus perhaps associated with temperature elevation. He believes that it is characteristic of *melitensis* infection since it has rarely been encountered in *suis* and *abortus* infections. The granules are similar in size to *Brucella* and stain similarly.

**Agglutinins**—The blood also usually shows the presence of agglutinins and sometimes of other specific immune bodies, as haemolysins. The agglutinins may appear in the blood by the fifth or sixth day after the onset of the fever and can often be demonstrated in very high dilutions. In other instances they may not be demonstrable until after the tenth day of the disease. The reaction may in some cases be present in the blood long after recovery from the disease.

Hardy examined from 45 of his patients blood sera collected 12 or more months after the illness had been first diagnosed. Of these 15 failed to show an agglutination in dilutions of above 1:10. In 5 other cases the serum became negative in from 3 to 9 months. 30 still showed agglutinins in titers of 1:40 or higher after 12 months but in 29 of these the findings indicated a marked reduction of titer. The thirtieth was a case of prolonged infection. Three men had persisting titers of 1:80 and 1:160 for several months. Hardy concluded that on the whole the tendency seemed to be for specific agglutinins to disappear rapidly following clinical recovery from undulant fever. In Keefe's patient the titer fell from a maximum of 1:20 480 during the disease to 1:30 four months after recovery. In one of Kern's cases with a maximum titer of 1:5120 it fell to 1:640 a week after defervescence. In a study of the reaction in 35 cases occurring in the United States Kern found that the highest agglutination was in Keefe's patient (1:20 480) the lowest (1:30) in a patient from whose blood serum a strain of *Brucella abortus* was isolated.

On the other hand infection may occasionally occur without any production of demonstrable agglutinins and this has sometimes been found to be the case even when the organism has been isolated from the blood. Archibald has recently reported a case of this nature with a negative agglutination. The case resulted fatally and at autopsy the organism

performed on patients with undulant fever in which the pathological examination revealed no evidence of any active inflammatory processes in the appendices or gall bladders. Milkoper reported upon a case in a boy of 11 years in which there was marked abdominal tenderness over McBurney's point and rigidity of the abdominal muscles. The appendix was found acutely inflamed and was removed. While after operation the abdominal pain disappeared the fever continued with daily fluctuations and an agglutination test for *Brucella abortus* was then made and was positive in a dilution of 1:320. Hardy also notes that abdominal pain was definite and severe in 70 per cent of the Iowa cases, sometimes continuous and sometimes cramp like. The localization was inconstant, appearing in some in the right lower quadrant or in almost any region. Hardy also emphasized that this symptom must be particularly borne in mind as it has led to erroneous diagnoses and to needless and often harmful surgical procedures.

Constipation is usually present. Hughes observed it in about 81 per cent of the cases which he described. It is often accompanied by flatulent distension and discomfort. It occurred in one half to two thirds of Hardy's cases, in which its degree paralleled the gravity of the infection. Simpson noted that the outstanding feature of the gastrointestinal effects of the disease was constipation, which was present in two thirds of his cases. Generally speaking diarrhoea is uncommon. However in the malignant type of the disease both Hughes and Bassett Smith say that it may be present with bilious stool. Hughes found it present in 4 per cent of his total cases. Seard and Lucas have also reported cases with diarrhoea. Diarrhoea was absent in Simpson's cases. With reference to this symptom Hardy says that a specific diarrhoea rarely if ever occurred, while in Kern's series diarrhoea occurred in 2 and alternated with constipation in others.

Intestinal haemorrhage has been referred to by Bassett Smith, Modinos, Lagnifoul, Arnal and Roge, Bousfield and Ivansson. Several of the cases terminated fatally. In some instances with intestinal haemorrhages in which at necropsy ulcerations of the small intestine were present, a diagnosis of concurrent infection with typhoid fever or tuberculosis of the intestines has been suggested. Griffin has reported the only instance in this country where there was a suggestion of intestinal ulceration, and this evidence was obtained particularly by roentgen ray examination. Simpson notes that meteorism, which is common in typhoid fever, is rare in undulant fever and was the source of complaint in only 4 of his cases. Sargent has reported a case of undulant fever which was complicated by ulcer of the stomach with haematemesis. Ascites has been noted by several observers in cases which have died with complications.

**Spleen**—The spleen is almost always swollen and it is often tender on pressure in the early stages of the disease. There may be a dull aching pain in the left side under the ribs. The spleen is frequently palpable below the costal margin by the end of the first week. In advanced cases which have lasted more than several months the condition of the spleen may come to simulate that observed in splenic anaemia.

The size and changes in the spleen have been fully discussed under the morbid anatomy. Bassett Smith, Sacquépée, Violle and Manson Bahr in their clinical descriptions of the European cases all emphasize enlargement of the spleen. Bassett Smith says that it is always enlarged, and Violle states that it is easily palpable in the majority of the cases. Manson Bahr believes that enlargement of the spleen should be considered a prominent factor in diagnosis. In the United States in Kern's, Hardy's and Simpson's series the spleen is noted as enlarged or palpable in one third of the cases. In 4 of Simpson's cases the spleen reached to from 3 to 8 cm. below the costal margin, in the remaining cases (when enlarged) it was palpable on deep inspiration at the level of the rib margin or just above it. Hardy also found that marked enlargement of the spleen is rare.

The lymphatic glands of the neck and groin may show hyperplasia, but no suppuration occurs. Simpson observed no case of generalized lymphadenopathy. However adenopathy was present in 2 of Kern's cases reported by Kern. In one the

and Scott and Saphir, and Jenkins in the United States have reported cases in which pleurisy, either dry or with effusion was present

Halbron found that in some cases the symptoms of pleurisy were apt to recur with the relapses and to coincide with the enlargement and volume of the spleen. The pleural symptoms occurred particularly at the base of the lung which suggested that there might be some extension of the infection from the spleen. Hughes found that when pleurisy was present it usually affected the left side. On the other hand Pisani found the pleural pulmonary symptoms occurring more particularly on the right side near the pulmonary apex. In a number of cases the rales in the lungs, high temperature and night sweats have led to the diagnosis of tuberculosis. Bassett Smith points out that cases with a hectic temperature, sweats and pulmonary signs have often been diagnosed as Mediterranean phthisis. Coffin and Famulener have reported a case in the United States in which night sweats, glandular enlargement and slight cough suggested tuberculosis. One of Hardy's cases was also diagnosed as miliary tuberculosis. In many of the cases with pulmonary symptoms the coexistence of an old tubercular infection should be considered. Such an infection might very well increase in severity owing to the debilitated condition of the patient. Pisani, Terzani, Sappa and Bethoux have emphasized the occurrence of this pseudo tubercular form of undulant fever. The disturbances consist of simple catarrhal pleural pulmonary congestion often associated with a bloody sputum and occasionally with a general haemoptysis. Pisani found this complication in 15 cases of a total of 75.

It seems probable that *Brucella* has not been isolated from the sputum even in cases with pulmonary symptoms. However there have been several unconfirmed reports of such isolation.

**Digestive System**—The appetite and power of digestion decrease in proportion to the severity of the disease. Anorexia is usually present while the fever is high but in the mild or prolonged cases with intermittent pyrexia the patient may crave and partake of more food than he can properly digest.

Anorexia was present in three fourths of Hardy's patients and in one half of Simpson's. The breath is usually foetid and patients complain of a disagreeable taste in the mouth. The tongue becomes coated with a yellowish white fur early in the disease and in severe cases it sometimes later becomes red at the tip and edges, tumefied, dry and brown in the center with sometimes patches of denuded surface epithelium. Poston and Menefee (1938) have reported a case with primary oral lesions resembling somewhat thrush infection in which *Brucella* was isolated from the ulcerated mouth lesions. Signs of gastric and more rarely of intestinal catarrh may be present in the early stages when occasionally nausea or vomiting occur. In the malignant case vomiting may sometimes be severe. Castellani emphasizes both the severity and persistence of this complication in such cases. Hardy found nausea alone present in 8 per cent of his cases and in association with vomiting in 13 per cent. The digestion is usually impaired and the epigastrium is tender on pressure. Tenderness in the iliac region usually is not elicited and is not so frequently encountered as in typhoid fever.

**Abdominal pain** was observed as a major complaint by Simpson in 16 of his 20 cases. This symptom was most common early in the course of the disease. In 7 of the cases the pain was located in the epigastrium, in 4 it occurred in the right upper quadrant while in 5 it was most marked in the right lower quadrant. Appendectomy was performed in 4 cases in which there was sudden development of right lower quadrant pain accompanied by fever. In one instance gangrenous appendicitis developed during the third week of illness. In 3 other cases normal appendices were removed. In these the surgeons requested agglutination tests for undulant fever after operation in their effort to determine the cause of the abdominal pain. In one case cholecystectomy was contemplated because of the development of sharp upper right quadrant sweats. Simpson mentions in addition 12 appendectomies and 2 cholecystectomies which were

tion of *Brucella* to abortion is discussed under PATHOLOGY. *Brucella melitensis* has been shown to remain for long periods in the vagina. Wainwright has reported the removal of a cystic ovary from which a pure culture of *Brucella melitensis* was obtained six years after onset of the disease. Kristen and Holm and Amoss have also recently isolated *Brucella abortus* from ovarian cysts. The action of the toxin on the mammary gland is frequently evident and in nursing women the milk usually dries up after a few days even when mastitis does not occur. While *Brucella melitensis* has often been found in the milk and nursing infants may be infected from the mother it is unusual to find the disease in children under one year. Tomaselli and several other observers believe that suckling infants acquire a certain amount of immunity. Calves also display a certain amount of immunity to infection.

The urine frequently shows a trace of albumin and in severe cases Bessis and Schöhl have each found albuminuria well marked. The urine is usually scanty and high colored in those cases where much diaphoresis has occurred. Several instances have been reported in which the disease was complicated by glomerular nephritis. Pickwick has recently reported a case in which considerable albumin persisted even during early convalescence: numerous hyaline and finely granular casts and an occasional red blood cell were present. In 13 of Kern's cases in which the urine was examined a trace of albumin was found in 4 instances, casts were found in 4 and pyuria was present in one. Since the majority of cases of undulant fever show no marked albuminuria when such a condition is present it suggests that it is not directly dependent upon the *Brucella* infection.

In some of the fatal cases subacute chronic nephritis also has been present. Baas and Schöhl have each reported a case complicated by uraemia. Bassett Smith mentions also that haematuria may occasionally occur and Poddighe has recently reported a case in which this symptom was prominent. Profuse haematuria appeared first on the ninth day of illness and the blood continued to be present for 40 days. Fever continued on and off for 5 months. The organism was isolated from the blood in this case. Castellani mentions that bile is sometimes present in very severe cases. Kern noted urobilinuria in one case. Hardy thought that in some of his cases the presence of numerous pus cells in the urine indicated either secondary infection or localization of the specific infection in the genito-urinary tract. A few were first treated as cases of cystitis or pyelitis. Burning pain on micturition or frequency of urination though transient in nature occurred in 11 per cent of the cases. In none there was a persistent but possibly unrelated pyelitis.

*Brucella* has frequently been isolated from the urine in human cases of the disease. However the organism is eliminated very irregularly. In 8 cases in the United States in which cultures were made from the urine the organism was isolated from 2.

**Nervous System.**—The implication of the nervous system has been emphasized. A number of investigators including Hughes Eyre, Bassett Smith, Gentry and Roger have all pointed out that the organism and its toxins appear to have a selective influence upon the nervous tissues. This neurotropism is more particularly peripheral but may be also central. Accompanying the fever headache pains in the limbs, general fatigue, despondency and insomnia are particularly common in the early stages of the disease so during its course. In Hardy's cases insomnia was experienced in 50 per cent during the height of the disease. Later there may be a hypersensitive state of the nervous system characterized by marked restlessness, irritability or grave apprehensions and neuralgic pains particularly in the muscles, subcutaneous fascia or about joints. Delirium or hallucinations may also occur. In a case reported by Broc and Bonan the patient remained delirious for 8 days without cessation night and day. She eventually made a good recovery and had a relapse of the fever one month later. Delirium occurred in 6 per cent of Simpson's cases. In severe infections more marked nervous symptoms may also be observed as the result of irritative lesions of the central nervous system and peripheral nerves and bulbar symptoms and those of meningitis and neuritis may occur.

Various forms of neuralgia have been observed in from 50 to 75 per cent of the cases and make their appearance particularly when the disease is well advanced. While almost any nerve may be involved the sacral sciatic, intercostal and perineal are more

lymph nodes were generally enlarged pea to nut size and in the other the cervical glands alone were involved. Manson Bahr in the report of 6 cases found the cervical glands enlarged in 3 and the inguinal in 3 while in 2 the symptoms were referred to the intra abdominal glands at the portal fissure which he says if unrecognized might lead to the suspicion that the patient was suffering from some affection of the gall bladder. Giordano and Sensenich found the axillary glands enlarged and tender in one of their patients a veterinary surgeon. They thought the lesion could be attributed to repeated infection from operating wounds sustained in handling infected cattle. Apparently no bacteriological examination of the axillary glands was made. Castellani mentions that inflammation of the parotid gland sometimes occurs. Amoss (1936) reports that a culture from suppurative cervical lymphadenitis simulating tuberculosis infection yielded a culture of *Brucella*. The isolation of *Brucella* from the lymphatic glands in Hodgkins disease is discussed under Diagnosis p. 798.

**Liver**—The liver may be more or less swollen and tender on pressure. In Simpson's series it was noted as palpable below the costal margin in 4 instances. Hardy also found the liver occasionally definitely enlarged. Giordano and Sensenich found the liver definitely enlarged in 2 of 35 cases. De La Chapelle has reported a case in which it was considerably enlarged, the lower edge reaching almost to the umbilicus. Hegler has reported 2 fatal cases in which atrophic cirrhosis of the liver complicated the disease. Jaundice has been noted in rare instances and cholecystitis has also been reported in which pure cultures of *Brucella melitensis* were isolated from the gall bladder by Bull and Gram. Amoss and Poston, Gilbert and Coleman and Giordano and Sensenich.

**Genito urinary System**—Orchitis is a not uncommon but a variable condition in undulant fever. Kern in his study of 26 cases says that it has not been observed in human *abortus* infection. However Hardy found it in 4 and Simpson in 16 cases. It is more commonly unilateral than bilateral and often very painful but it usually only lasts a short time and does not suppurate nor end in atrophy of the testicle. If there has been much effusion into the tunica vaginalis the organ may remain for a time somewhat enlarged and tender. In other cases there may be neuralgia or an inflammatory epididymitis without visible orchitis. Simpson pointed out that in the study of his cases there was convincing evidence that *Brucella abortus* exhibited the same selectivity for the genital tract of human beings as it does in cows and bulls, painful swelling of the testes being a prominent feature of the disease in 16 cases. He points out that evidence was also found in 3 of the Dayton patients of seminal vesiculitis, prostatitis, epididymitis and orchitis in which the history and laboratory examinations eliminated gonorrhea from consideration. The sera of these 3 men agglutinated *Brucella abortus* in dilutions from 1:160 to 1:640. The organism was recovered in one case from a draining sinus tract which extended from the globus major of the epididymis through the scrotal wall. In a case reported by Leavell there was epididymitis and the epididymis was removed. However cultures from it and from the seminal fluid were negative for *Brucella*. Bevan has reported an instance in which there was seminal vesiculitis with blood stained semen and Manson Bahr has noted the occurrence in the male of frequent intermittent haemorrhages from the urethra.

In the female *ovaralgia*, *ovaritis*, *menstrual disturbances*, *mastitis*, diminution of the milk secretion and tendency to abort have been called attention to by many writers. Lafont has emphasized the fact that abortion and premature labor are favored particularly during the periods of high fever and he calls attention to the fact that the infection can pass through the placenta. Williams has reported a case in which infection of the child *in utero* occurred apparently through the placenta but in spite of this complication the pregnancy went on to term with the delivery of a living child. However during pregnancy there is increased danger from haemorrhage both on account of the changes in the blood and the loss of muscular tone in the uterus. Nevertheless in *Brucella abortus* infection abortion in women seems to be less common than in cows. Abortion as a complication is not noted in Kern's American cases. Hardy observed but one infection during pregnancy the condition proceeding normally. Simpson and Frazier however encountered 5 cases in women who had repeatedly aborted and who presented no clinical or serological evidence of syphilis whose blood gave a very high agglutination test for *Brucella abortus*. Other evidence of the relation of the infec-

prostration irritability and puerilism are symptoms which have been frequently noted

**Joints**—Arthralgia is one of the cardinal symptoms of undulant fever as it occurs in Europe having been noted in approximately 40 per cent of the cases. The joints become swollen hot and extremely painful but without redness of the overlying skin. These symptoms may occur as an acute infection sometimes early in the disease but more often as a subacute one in the later stages.

In the United States Simpson noted tenderness or pain in the joints in 31 of his cases and in 4 the presence of migrating pain in the larger joints led to an initial diagnosis of acute rheumatic fever. Hardy also observed that tenderness in the region of the joints was not unusual and that pain on active motion was a rather frequent complaint. However hydrarthrosis or swelling of the joints was unusual in his cases occurring in less than 2 per cent. In 5 of Kern's collected cases the joint symptoms were prominent. The lesions are often transient. An acute or subacute effusion may appear usually in one joint at a time and it may be exceedingly painful on the slightest movement. Within 24 hours however the tender swollen condition may disappear but the next day another joint may be affected. Sometimes the pain and swelling may remain in the joint for several days. The hip knee shoulder and ankle joints are most frequently involved but almost any joint may be affected. When the sacro iliac or vertebral joints are involved the condition is usually especially painful. In addition to sacro iliac involvement Roger has also observed spondylitis in 5 cases. The 2 chief symptoms were lumbar pain and stiffness of the spine. The pain was severe especially at night radiating towards the thigh sometimes simulating sciatica. There were symptoms of pyramidal irritation with changes in the reflexes. Simpson and Baker have each reported a case in which intermittent hydrarthrosis was present and in each instance *Br. cella* was isolated from the joint fluid. In Baker's case the process of intermittent and alternate swellings of the knee joints continued regularly for nearly 7 months and was accompanied by irregular fever. Thick fluid was drawn off from the knee joints on several occasions and *Brucella abortus* as cultivated from it as well as from the blood. While neither suppuration or ankylosis usually ensues the stiffness may remain for several weeks or months. In 3 of Hardy's cases it persisted for more than 6 months while in 14 patients during convalescence mild or moderately severe joint pains occurred. In a few instances cold abscesses have formed in the chordal and sternal joints. In one of these Kennedy obtained *B. cella melitensis* in pure culture.

Disease of the bones is evidently very rare. Burnet Brun and Boan have reported a case of suppurative osteitis in a patient in which the symptoms had relapsed 3 times at intervals of 7 to 20 years and from which *B. cella melitensis* was isolated. In this case the suppuration had the character of a cold abscess. Edwards (1937) has noted localized abscesses in the bones.

Burnet in the experimental infection of guinea pigs with *Brucella melitensis* found that they frequently developed arthritis. Radiography showed rarefaction and demineralization of the bones evidences of osteomyelitis and involvement of the surrounding soft parts. Hardy has also mentioned that joint and bone lesions occur in guinea pigs inoculated with cultures of *Br. cella abortus* var *suis*. Weil has reported a human case with arthritis and osteitis of the right foot in which the roentgen picture showed bony atrophy and blurred contours of the joints between the cuneiform and metatarsal bones. The swelling of the dorsum of the foot gradually subsided and normal conditions were eventually restored.

**Skin and Appendages**—There is no characteristic rash in undulant fever. Nevertheless various eruptions have been recorded by a number of observers. The occurrence of petechiae or purpuric lesions in the skin or patches of subcutaneous ecchymosis have been frequently noted.

commonly affected. The pains are sometimes very severe. Bassett Smith mentions a case in which the patient was almost completely helpless, unable to move in bed or feed himself, and where intense hyperesthesia of the feet aggravated the discomfort. Sometimes these neuralgias persist during early convalescence, being often localized in the intercostal nerves and the sciatic, but occasionally in the occipital. In 29 per cent of Hardy's cases, pain occurred in the back of the neck and was severe in one fourth of these. The stiff neck and muscular soreness with pain, he observed, was occasionally the first symptom of the disease. Rarely it was so intense as to lead to a suspicion of meningitis. Hughes called attention to localized pain in the plantar region. The patellar and plantar reflexes are generally increased in the early stages, but in chronic cases they may be decreased, and other symptoms of peripheral neuritis, as tingling and prickling sensations or hyperesthesia, are sometimes present. Symptoms suggesting neuritis were reported in 3 of Hardy's cases. The neuralgias may terminate by a paralysis or muscular atrophy.

Sometimes there may be partial paralysis of a group of muscles which, however, does not remain permanently. Hughes and Bassett Smith have both reported slight paralysis with loss of reflexes, and the latter has described a case with ascending neuritis going on to transverse myelitis with complete paraplegia and all the attendant symptoms. Johnsson has recently reported a case in which there was well marked paralysis, muscular atrophy, and sensory disturbances in which *Brucella* was isolated. The paralysis and atrophy involved especially the muscles of the right shoulder and induced a nearly complete paralysis of the right scapulohumeral articulation.

Cantani and Grocco have noted bulbar signs such as disturbances of cardiac and respiratory rhythm, with uncontrollable vomiting not associated with a loss of consciousness. In other cases they have observed mental symptoms, with semi mania and periods of unconsciousness followed by a state of marked asthenia.

Henry Roger has also described several cases which showed some form of nervous symptoms of central origin. The first of these had occasional aphasia followed by facial paralysis, while the second had very marked wasting of groups of muscles, difficulty in walking, and vertigo. A third showed paraplegia with involvement of sphincters and positive Babinski sign. In 3 of his other cases of undulant fever the spinal cord was involved, and in 2 of these there was involvement of the nerve roots with marked paralysis of the lower limbs without involvement of the sphincters.

In young children the nervous system would appear to be often more markedly affected than in adults, and a number of cases of meningitis in children have been reported. DiChristina and Maggiore observed that in the meningitic type there was a leucocytic proliferation, the meningeal encephalitis showed a preference for the Rolandic area, and in these cases convulsions and spastic paralysis might occur.

Lemaire, who called attention to the frequency of meningeal symptoms in adults, pointed out that pathological changes in the cerebrospinal structures have rarely been demonstrated. Smith and Poston (1936) reported a case of *Brucella* meningitis with recovery. The occurrence of meningitis is further referred to under *Pathology*. In a case which Lemaire reports, the first marked symptoms were those of meningitis. The case was at first thought to be one of influenza, and then of tubercular origin, but later *Brucella melitensis* was isolated from the cerebrospinal fluid, demonstrating its true nature. Both Violle and Lemaire point out that in cases with meningitis the cerebrospinal fluid may show an increase in albumin and sugar content, while Lemaire remarks that its cellular reaction is lymphocytic, similar to that observed in syphilis and tuberculosis. Roger found in the examination of the cerebrospinal fluid of 4 cases that it was normal in 2, while in the other 2 there was an increase in the albumin, which was relatively large in comparison with the lymphocytosis. Suarez found in one case that there was a well marked albuminal cytological dissociation, there being only 0.3 gm. of albumin with 60 cells to the c mm.

As a rule, the nervous symptoms in undulant fever are only temporary, complete recovery usually following. During early convalescence psychic phenomena are sometimes quite disturbing, and changes in memory

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Bassett Smith states that in the cases which have been observed in the British navy since 1919 there has been a greater tendency to purpuric and hæmorrhagic complications particularly among the older patients. Castorini reported a case in which the punctiform subcuticular hæmorrhages reappeared with the relapses of fever. In Rudduck's case crops of petechiae occurred after each wave of fever. The occurrence of rose spots or lesions resembling rose spots have been called attention to recently by Hyat Gouget Bancelhon and Custer in Europe and by Kern Simpson and Duffie in the United States. Simpson notes that a skin eruption occurred in 10 of his cases. The lesions were generalized and took the form of small macules in all but one instance, in which the eruption was maculopapular. In 3 cases the lesions were most prominent on the abdomen and simulated the roseola of typhoid fever.

The sweats which are so characteristic of the disease have been particularly referred to under the heading of fever. The sweat has often been described as having a distinctive and disagreeable odor. As might be expected in a disease in which there is such frequent sweating sudamina are frequently abundant. With the development of the anaemia the skin shows more or less pallor. The hair becomes brittle and often falls out during convalescence but generally grows again. Shaw has noted in some cases that the nails become striated longitudinally.

Huddleson and Johnson state that for several years veterinarians have informed them that a peculiar erythema develops on the skin of their arms following contact with the vagina of certain cows, particularly those who have aborted. In one type light red irregular blotches appeared on the skin, or the entire surface of the forearm became light red. In the second type the rash appeared as small discrete elevated reddish papules which were more widely separated than in the first type. The accompanying itching and burning was very severe. The papules often persisted for 3 or 4 days their color changing to dark brown. There was no exudation or desquamation.

Huddleson and Johnson believe that the reaction elicited by means of the intradermal test in those veterinarians showing skin hypersensitiveness is quite conclusive evidence that this symptom is due to *Brucella abortus* protein against which they have in some manner become hypersensitive. Further investigation on the nature of this cutaneous rash described would seem to be desirable.

#### COMPLICATIONS AND SEQUELAE

The most common pulmonary complications are bronchitis bronchopneumonia and pleurisy. Those of the circulatory system are endocarditis myocarditis and hæmorrhages from the mucous membranes and in the skin. These conditions have all been described in detail under the discussion of the circulatory and respiratory systems. It is not clear whether a primary acute endocarditis has been produced by *Brucella* or whether in the cases of undulant fever in which endocarditis has been present some other microorganism has not been primarily responsible for the lesion. The nervous complications are very common but as a rule are only temporary and in a large percentage of the cases complete recovery follows. Particularly frequent are the neuralgias and mild

forms of neuritis. The tendency to abortion and premature labor in pregnant women, ovarian pains, menstrual disturbances and mastitis and in men orchitis as well as nephritis have been discussed under the heading of Genito-urinary System. Disturbances such as splenic and hepatic enlargement, swelling of the joints and other conditions which are rather phases of the disease have also been referred to under Symptomatology. Bassett Smith remarks that he has frequently noticed in patients debilitated from severe and protracted attacks that a tuberculous infection has followed and that the marked anaemic condition and general debility produced by the disease no doubt lowers the resistance and makes the patient very prone to contract other infections. When death occurs it is usually from sudden hyperpyrexia, some pulmonary or cardiac complication or occasionally from exhaustion or haemorrhages. The most common of the sequelae are the general debility, tendency to emaciation, anaemia, rheumatic like pains, persistent neuralgias, particularly sciatica and occasionally neuritis. Hardy reports that in a few of his cases mental depression or nervous irritability was a serious and prolonged sequel. Manson Bahr mentions that muscular wasting and laxity of the ligaments of the knee and foot may bring about a considerable degree of disability during convalescence.

### DIAGNOSIS

**Laboratory Examinations**—As the clinical features of undulant fever are not pathognomonic, the diagnosis must be based upon laboratory examinations. Without these the disease may be confused with a number of febrile conditions such as typhoid or paratyphoid fever, certain forms of tuberculosis, infectious arthritis, streptococcal infections, pyelitis, kala-azar or even sometimes with chronic malaria or influenza. Bassett Smith believes that the only efficient means of diagnosis is by laboratory methods and in this opinion the writer concurs. The following procedures have been employed in the diagnosis: (1) bacteriological examination of the blood, urine and faeces or other pathological material; (2) agglutination test; (3) complement fixation test; (4) intradermal and phagocytic tests; and (5) inoculation of animals.

**Isolation of the Organism**—By far the most reliable procedure is the cultivation of the organism from the blood or enlarged spleen. However, splenic puncture may not always be justifiable, as fatal haemorrhage might occur if the spleen is very soft. Eyre, however, encountered no unfavorable results in splenic puncture. Blood cultures should be taken preferably at the onset of a febrile paroxysm. The organisms are present in the blood early in the disease in many of the cases infected with *Br. melitensis*, less often in those infected with *Br. abortus*. The organism has in some instances been cultivated from the urine, but as it is frequently absent or present only in very small numbers, unless a positive result is obtained the procedure is of little value for diagnosis.

*B. Ha.* has been isolated from the urine in cases with initial symptoms of cystitis and renal tuberculosis and from the discharges following gonorrhoea. A 50 cc. sample

of catheterized urine should be first thoroughly centrifuged and cultures made from the sediment upon crystal violet agar (0.5 per cent). The dye dilution in this media should approximate 1:700,000. The presence of this amount of the dye inhibits the growth of the majority of Gram positive organisms but does not inhibit the growth of *Brucella*. In a few instances the organism has been isolated from the faeces from effusions in the joints from subcutaneous abscesses from the gall bladder and even from the milk. Baker isolated *Brucella* from the joint fluid of a double recurring hydrarthrosis of the knees the original attack occurring 17 years previously. There are also isolated reports of its cultivation from the epididymus tonsils sputum cerebrospinal fluid and pleural fluid and bile by duodenal drainage before operation. Amoss (1936) isolated *Brucella* from a case of chronic peritonitis with tuberculous salpingo-oophoritis and the organism also was recovered from a small cyst on an otherwise normal ovary.

**Other Tests**—Next in importance to the isolation of the organism is the agglutination test. The complement fixation test has also been employed for diagnosis but apparently has no advantages over the agglutination test. The intradermal reaction in some instances may also give additional data in connection with the diagnosis and animal inoculations may be of some assistance in isolating the *Brucella* from material where other microorganisms are present or in determining the virulence of the different strains.

**Blood Culture**—To avoid the inhibitory effect of the patient's serum a small amount of blood should be placed in a large amount of liver broth.

Huddleson (1940) has found bacto tryptose broth to be the most satisfactory medium the bacto tryptose being prepared by the Difco Laboratories. The pH of the medium is adjusted to 7.2. After sterilizing at 15 lbs. pressure the final pH should be 6.6 or 6.8. Sodium citrate 1 per cent is added to serve as a blood anti-coagulant. The medium is distributed in 50 cc. serum bottles in 20 cc. amounts and is inoculated with 2-5 cc. of blood directly the bottle being shaken vigorously to prevent clotting of blood. In a liquid medium in the presence of blood he has found that all 3 species of *Brucella* whether aerobic or anaerobic appear to grow better when the CO<sub>2</sub> tension inside the culture bottle is increased approximately 25 per cent. It is advisable to introduce the CO<sub>2</sub> into the bottles before inoculating the blood. At the end of each fourth day the culture should be mixed by shaking the bottles. Five tenths (0.5 cc.) of the culture is then removed and added to a bacto tryptose or a liver agar Petri plate and the preparation incubated under 10 per cent CO<sub>2</sub> for 4 days. If no growth is obtained from the blood culture within 20 days it may be discarded. Any suspicious colonies which develop on the plate should be transferred to agar slants and their identity confirmed by other cultural and serum reactions.

The isolation of *Brucella* from the blood during life is clearly the most satisfactory way of establishing the diagnosis and as Wilson has recently emphasized every effort should be made by repeated blood cultures if necessary, to attain this end. Success however, has not been by any means uniform. In undulant fever due to *Brucella melitensis* it has been frequently possible sooner or later to recover the infective organism if the blood has been taken during the febrile stages of the disease and particularly at its height.

Wattan Lerner reported that blood cultures were positive in about 80 per cent of the cases.

Huddleson (1940) cultivated the organism in 37 cases out of 38 that were febrile but only in 5 of 17 cases that were afebrile. Eyre had 152 positive results in 235 cases. Shaw 68 positive results in 103 cases. Cilmour 38 positive in 45 and Bassett Smith 16 positive in 24 cases.

In cases of supposedly undulant fever where the infective organism has been presumed to be of the bovine type attempts to isolate *Brucella* have often failed. Doubtless in some of these instances the disease was not undulant fever as a careful study of some reports would suggest. Failure also may be due to the use of unsuitable culture media or to the absence of proper gaseous tension in the retainer of the culture. However several observers have reported that it is much more difficult to obtain cultures of *Brucella abortus* from the blood than *Brucella melitensis*. Kristensen and Holm isolated *Brucella abortus* from the blood in 23 instances or about 65 per cent of their cases.

In one instance they isolated this organism from an ovarian abscess and once from the placenta in a case of human abortion. Simpson remarked that in his hospitalized patients he succeeded in recovering the organism from 7 of 9 patients. In one of his cases the organism was finally recovered from the blood after 6 negative results. Carpenter and Boak also reported that they were able to grow cultures of *Brucella abortus* from about 6 per cent of the blood samples submitted for pathological examination. Hardy reported that *Brucella* was isolated from the blood stream of 48 of the patients in Iowa. In one case he noted that both the *abortus* and *swiss* varieties were obtained from a single culture. The difficulties in differentiation of these strains have already been discussed in this article. Hardy also points out that his findings which were confirmed by Huddleson on all but a few of the recent isolated strains showed 35 of the variety *swiss* and 14 of the variety *abortus*.

Teani believes that the organism can be more often cultivated from the blood clot than from the serum itself. Hardy however found that efforts to isolate the organism from the clots of blood were uniformly negative. Guinea pigs inoculated with the clots of 35 blood specimens giving positive serum agglutination of *Brucella* failed in every instance to develop infection though from some of the patients positive blood cultures were obtained later from the blood serum.

The organism is frequently present in the blood in the early stages if fever is present but it may be isolated also later in the disease. For example out of 80 successful blood cultures performed by Duffau 30 were obtained in the first 15 days of the disease, 15 in the third and fourth week, 10 in the second month, 3 in the third month and 2 during the sixth month of the disease. Bassett Smith isolated the organism in one case on the 142nd day. Angelis after one year in a case with intermittent pyrexia while both Eyre and Gilmour have reported its recovery from the blood as late as the 300th day of the disease.

**Cultures from Faeces.**—Amoss and Poston have reported the isolation of *brucella* 78 times from the stools of 6 different patients. The essential procedure of their method was to clump the organisms present with immune serum added to the stool suspension and to concentrate by differential centrifugation.

Loops of the final precipitate are inoculated on stock eosin methylene blue plates made with meat extract agar adjusted to pH 7.4. Four plates were seeded in each instance and incubated at 37°C. 2 plates in 10 per cent carbon dioxide and the others aerobically in the incubator. The delicate colonies which appear are transplanted into broth and identified. Amoss and Poston have isolated both *Brucella abortus* and *Brucella melitensis* from the stool by this method. Obviously it is only in some instances that *Brucella* are present in the stools even during the acute stage of infection.

**Agglutination Test**—A number of investigators particularly in the United States have found the agglutination test the most valuable procedure in the diagnosis of the disease, but great care must be taken to avoid errors. The agglutinins frequently appear in the blood serum of undulant fever cases as early as the fifth to tenth day and then usually persist in the blood for a long time. By the end of a week or ten days the reaction frequently occurs in dilutions of 1:100 or 1:1000 though this is not always the case. Simpson reports that in 7 of his patients the agglutinins did not appear until the third or fourth week of illness. The time after the attack that the agglutinins are still demonstrable in the serum varies greatly. In the case of the writer, who contracted the disease in the Philippine Islands the blood serum showed a positive agglutination in dilutions as high as 1:100 or 1:200 for approximately a year. However in some instances the blood serum of individuals has been said to show a positive reaction in dilutions of 1:50 to 1:100 for from 3 to 10 years after the attack of the fever. Carpenter and Boak tested the blood serum from 3 patients monthly for approximately 2 years and at the last examination it still showed comparatively high titers. One serum agglutinated the *abortus* antigen in a dilution of 1:45 while the other two showed agglutination one in a dilution of 1:405 and one in 1:1215. Kristensen and Holm found that generally the specific agglutinins disappeared within a few months to a year after recovery from the disease. Hardy reports the examination of blood sera from 45 of his patients collected 12 or more months after the illness has been diagnosed. Of these 15 failed to show agglutination in dilutions above 1:30. In 5 other cases the sera became negative in from 3 to 9 months while 30 still showed agglutinins in titers of 1:40 or higher after 12 months.

**Technique**—The use of dry blood in performing the agglutination test is often unsatisfactory and more errors are likely to occur with it. The test is probably best performed by the macroscopic method in which graded quantities of the patient's serum are added to a suspension of *Brucella abortus*. The blood after being collected is allowed to clot, the serum removed and divided into two parts, one of which should be heated to 56 C to remove the nonspecific agglutinins. The reaction should be performed in a series of dilutions of 1:10 to 1:1000 or often higher. The bacterial suspension in saline solution of *Brucella abortus* should be prepared from a smooth stock strain of known agglutinability which has been grown on glucose agar for 48 hours. It may be killed by heating to 65 C for 30 minutes and made up by dilution into a stock antigen of definite turbidity standard as recommended by Evans. In performing the test 0.5 cc of antigen of a turbidity standard of 1:1000 is added to each tube containing 0.5 cc of the diluted serum, the final antigen turbidity being 1:500 as recommended by the American Public Health Association. Evans has laid emphasis upon a constant density of the antigen if the results of different observers are to be compared. The tubes are incubated in a water bath at 37 C for 4 hours then removed to an ice box and allowed to stand until the following day when readings are made. Only complete or practically complete agglutination should be reported as positive. Giordano and Ableson recommended that for antigen cultures grown in veal broth peptone be employed in preference to a sodium chloride suspension of agar cultures. Walsby, Lynch and Callan and a number of other bacteriologists in addition to Evans recommended a heat killed suspension of *Brucella abortus* for antigen particularly on account of the danger of laboratory infection. Carpenter and Boak however believe that a living antigen standardized to give a reading of 3.5 cm on the apparatus recommended

by Gates for standardizing bacterial suspensions is most satisfactory. The writer also believes that the living antigen is preferable to employ for the agglutination test with *Brucella*. He however hesitates to recommend it for use in public health laboratories where hundreds or even thousands of agglutination tests are being performed for diagnosis. Carpenter and Boak employ an automatic pipetting syringe known as a rheometer for the avoidance of laboratory infection. The serum is diluted directly in the antigen which eliminates adding the salt solution to the tubes and diluting the serum in the salt solution. Readings are made after 8 hours and after 42 hours in the ice box.

They point out that because *Brucella abortus* agglutinins are usually reciprocal with those produced by *Brucella melitensis* and occasionally with those produced by *Bacterium tularensis* the serum should be set up also with antigens prepared from these two organisms. Obviously a series of other control tubes should also always be prepared.

For the antigen employed it is important that a strain of *Brucella abortus* should be selected that has been recently isolated. Many strains after continued subculture in the laboratory have been found to lose their agglutinability. The discussion of the formation of rough strains has already been referred to as well as the disadvantages of their use. If the cultures are killed enough agglutinating suspensions may be prepared at one time to last for several months. For the simple agglutination test there would appear to be no necessity either in the United States or Great Britain to use more than one strain of *Brucella* (*Brucella melitensis* or *Brucella abortus*) since the bovine and porcine strains are usually agglutinated to a more or less equal degree with a serum prepared against any one of them and it has been pointed out that it is impossible to distinguish between these three types by direct agglutination. However Burnet in Lunis and Cerruti and Sollai in Sardinia point out that there are in those localities two distinct antigenic types of *melitensis* one *Brucella melitensis* and one *Brucella parameitensis* which necessitate the use of both these organisms in routine agglutination tests. In the United States Plastring and McAlpine have recently described a mutant or mucoid type of the *abortus melitensis* group simulating in some respects a *parameitensis* strain.

Huddleson and Abell have employed a rapid macroscopic method of agglutination in which a heated standardized suspension of *Brucella abortus* in 2 per cent sodium chloride solution is added to different amounts of undiluted serum on the glass cover of a specially lighted box. They believe that results obtained by this rapid method indicate that its accuracy and specificity are equal to those of any other method.

It is impossible to designate any specific serum titer for a positive diagnosis of undulant fever because no titer has been agreed upon by different bacteriologists. Many laboratories have regarded an agglutination in a dilution of 1:80 or above as diagnostic.

**Sources of Error**—In some cases in which the agglutination test results negatively the diagnosis of brucella infection cannot necessarily be excluded. A few instances (as noted) have been reported in which the agglutination test was entirely negative yet the organism was isolated from the blood during life or from the spleen at autopsy.

Carpenter and Boak noted that in 6 per cent of their cases of undulant fever in which the organism has been isolated from the blood the agglutination reaction was negative. In other cases of undulant fever the serum has sometimes not given an agglutination test in a dilution higher than 1:10. Bissett Smith reported 3 cases in which the limit of the agglutination titer was 1:10. In one of these cases the organism had been isolated from the blood during the same month. Evans mentions also a laboratory

infection and 3 other cases in which at one stage of the disease the highest titer of the serum giving the test was 1:10 but in which *Brucella melitensis* was cultivated from the blood. Bassett Smith considers complete agglutination at 1:30 as diagnostic. Evans regards complete agglutination in a 1:40 dilution or lower as suspicious and in dilutions of above 1:400 good evidence of undulant fever past or present provided tularaemia can be excluded. She further says that in regions where undulant fever is endemic the lower agglutinating titers of 1:5 to 1:10 would be regarded by most workers as sufficient to suggest an infection of undulant fever.

If however one relies for the diagnosis solely upon the agglutination test in these low dilutions frequent errors will unquestionably be made. In performing the agglutination test in undulant fever, it is well to recall that the reaction may sometimes persist for years after the original illness and that the individual in question may be suffering from another fever and still give this reaction.

For several years the writer has attempted to emphasize the difficulties and limitations in the employment of the agglutination test in the diagnosis of undulant fever as well as of tularaemia especially on account of the spontaneous and nonspecific agglutination that is particularly likely to occur in certain cultures of these organisms under a number of conditions not yet entirely understood. The spontaneous agglutination of at least certain strains of *Brucella* is likely to occur for example, in some samples of milk. Why this is so we are not yet able to explain but the reaction in some instances may render the agglutination test unreliable in relation to the demonstration of anti-brucella agglutinins in milk.

In testing cows and goats milk for the presence of agglutinins numerous controls must always be made. Sometimes the serum will give no agglutination reaction in lower dilutions and will react positively in higher ones. This phenomenon is due to the occurrence of proagglutinoïd zones, which are particularly liable to be found in *Brucella melitensis* sera.

Instances of this phenomenon have recently been reported in the United States by Carpenter and Boak, Simpson and Hardy, Henry and Traum. They found that in testing agglutination by the tube method formalized antigen has a tendency to intensify or cause proagglutination with human, bovine and porcine sera to such an extent that occasionally strong positive sera might be missed in routine testing while in the same sera tested with phenolized or trichresolized antigen this interference is absent or reduced to such a point that it is not misleading.

Coolidge believes that a passive absorption of *abortus* agglutinins may occur in man and appear in his blood serum after drinking infected milk. He fed cows raw milk with a serum titer of 1:40 containing *Brucella abortus* to 7 adults. After drinking the milk the serum of the individuals also had a titer of 1:40 but all of them remained entirely healthy. He concluded from this that passive absorption of the *abortus* agglutinins in the milk through the mucous membranes of the intestinal wall into the blood serum had occurred. Dooley (1932) found 41 per cent of healthy boys in a school with agglutination tests of 1:40 to 1:1200. Only two boys in the school had any clinical manifestations of *Brucella* infection. However, Carpenter, Boak and Chapman found that *Brucella abortus* agglutinins could not be demonstrated in the blood serum of 9 adults who were given to drink pasteurized milk containing *Brucella abortus* antibodies. They believe that the presence of *Brucella abortus* agglutinins in the blood serum is the result of an active production by living organisms which have invaded the tissues of the body and they have also interpreted Coolidge's result in this way.

In the very large number of agglutination tests which have been performed in the United States during the past two years a positive agglutination reaction with *Brucella* has been recorded in a number of instances in which the individual has shown no evidence of disease. Two opinions have been expressed in the interpretation of such reactions. Some observers suggest that in such instances the reaction implies that the individual has a latent infection and that the occurrence of the agglutinins is really the result of some infection with *Brucella*. In the absence of all clinical symptoms a former infection from which the patient has recovered is assumed. On the other hand other observers incline to the belief that at least many of these positive reactions in healthy individuals can be explained by the phenomena of nonspecific or spontaneous agglutination of the cultures employed. That nonspecific reactions of *Brucella* may occur in fairly high dilutions of normal sera and especially in that of certain other febrile diseases is well recognized. Nègre and Raynaud and others have shown that many sera will cause nonspecific clumping of *Brucella melitensis* and *paramelitensis* strains if the serum is not heated to 56 C for one hour. Therefore it is recommended in making the agglutination test that this heating should always be done with one portion of the sera and the test performed with it as well as with a non heated portion. However merely heating the serum obviously does not eliminate all nonspecific reactions. Burnet has called attention to the fact that *Brucella paramelitensis* is readily agglutinated in a dilution of 1:250 by normal human serum and that suspensions of it have a decided tendency to spontaneous agglutination. When this strain is inoculated into rabbits their blood serum only shows a low titer of agglutination in contradistinction to where *Brucella abortus* and *Brucella melitensis* strains are employed which produce a much larger amount of agglutinins.

The *paramelitensis* strains are common in Tunis. Both Failli and Burnet have shown that cultures of *Brucella abortus* and *Brucella melitensis* can be rendered thermoagglutinable by the action of both normal and immune sera as well as by the addition of certain antiseptics such as formalin iodine and even bile. By thermoagglutination Burnet implies an agglutination which occurs by heating a suspension of the microbes in normal saline in a water bath at 90 to 100 C for a time varying from a few minutes to one or even two hours. He selected 5 strains 4 *melitensis* and 1 *abortus* strain and rendered these thermoagglutinable in the manner described. Such strains then behaved as *paramelitensis* strains since they were now readily agglutinated by normal human serum and proved to be bad antigens when inoculated into animals. The *abortus* strain however was more stable than the *melitensis* strain.

Favilla emphasizes that one great difficulty in carrying out agglutination tests with *Brucella melitensis* is that reliable strains may not be on hand. Using the formula of thermoagglutinability he has found notably that the degree of thermoagglutinability inversely proportional to the degree of agglutinability by immune serum. He emphasizes that one should select for agglutination tests only those strains which show no trace of thermoagglutinability. The technique of selecting the strain he says is simple. Emulsions of the various strains are prepared in normal saline and heated in a water bath at a temperature of just over 80 C for 15 or 20 minutes. Emulsions are then examined by the naked eye to see if thermoagglutinability has occurred. A loopful is also examined in the agglutinoscope. All strains showing even a trace of clumping should be rejected and only the strain which gives homogeneous suspensions should be retained for use in agglutination tests. Traum and Henry have recently



emphasized the discrepancies which may occur in the agglutination tests when mutant types of *Brucella* are used as antigens

Plastringe and McAlpine have described a mutant type of the *abortus melitensis* group. This form which they described as mucoid is a capsulated cluster forming organism. Serologically it is less active than the normal type. It has pure antigenic properties and rabbits which have received injections showed the presence of agglutinins only after repeated large doses. It is agglutinated poorly by normal type antiserum and moreover exhibits marked spontaneous agglutinability.

Recently the experiments of Fitch have seemed to show that small amounts of agar have some influence on thermo agglutination of the organisms of the *Brucella abortus* group.

It is important to recall the report of Theobald Smith which called attention to the spontaneous agglutination of the organisms of the *Brucella* group through the action of agar. He pointed out that when growth from the agar surface is suspended in water normal saline or bouillon, *Brucella abortus* exhibits active Brownian motion. When the same growth is suspended in a small drop of condensation water from the same or a sterile agar tube the clumping is so prompt that all the bacteria appear in dense cloudlike masses no matter how quickly the slide is placed under the microscope. At first the clumping was referred to specific agglutinins in animal tissues placed in the tube but was soon found to be inherent in the agar itself. The clumping persisted in the condensation water of a culture consisting of 2 per cent agar only. Acid agglutination was eliminated inasmuch as the clumping took place in a neutral medium. It did not occur among bacteria from the sloped agar surface itself when they were suspended in bouillon normal saline or water.

Mallmann has reported that the serum of a *Brucella* infected cow will agglutinate suspensions of *Pasteurella borisepctica* in the same titer as *Brucella abortus* and will also agglutinate *Pasteurella aviseptica* and *Escherichia mallei* to somewhat lower titers than it agglutinates *Brucella abortus*. However the titers in all cases were well above 1:50. Similar results were also obtained with an immune *Brucella abortus* rabbit serum and an immune *Pasteurella borisepctica* rabbit serum which agglutinated *suisepctica* and *Brucella abortus* to full titer. An immune *Brucella suis* serum behaved in the same way as a bovine *abortus* serum. If such results are found to be common they will emphasize further the occurrence of nonspecific reactions in *Brucella* infections.

Some observers state that the serum of tubercular subjects sometimes has an agglutinating action on *Brucella melitensis* in comparatively high dilutions. However several investigators who have found agglutination reactions among patients in tubercular sanatoria have concluded that the patients were really suffering from undulant fever.

Amabile and Fici did not find that there was any definite tendency of the sera of tuberculous patients to agglutinate *Brucella melitensis*. Beilinger and Levin performed agglutination tests on 180 patients in a tuberculous hospital in Oregon and obtained positive agglutination in 43 cases. Twenty three reacted with a titer less than 1:50 the remainder to a titer up to 1:300 or over. In only 3 of the cases were the clinical symptoms of undulant fever present. Of the 2 cases that showed the highest titer one showed no clinical symptoms attributable to undulant fever while the other did. Three other patients showed symptoms that were too mild and vague for the clinical diagnosis of undulant fever. Giordano and Ableson obtained positive agglutination in 10 patients in a tubercular sanatorium but they remarked that in 6 of these patients the

agglutinins were present in dilutions of 1:20 or less and the diagnosis of tuberculosis in these had not been confirmed.

In a study of 150 cases of undulant fever Gilbert and Coleman found suggestive Widal reactions in 23 cases and definitely positive typhoid agglutination tests in 24 additional cases. Definite fluctuations in the typhoid agglutination titer were observed during the course of the disease.

Faecal examinations of these cases were negative for microorganisms of the enteric group. About one third of the group in which the Widal reaction was positive had never had typhoid or paratyphoid vaccine. Their investigations they believe demonstrated that agglutination of the *Bacillus typhosus* will occur with the blood serum of certain cases of undulant fever in patients that have never received typhoid vaccine nor to their knowledge had typhoid fever and that fluctuation of the agglutination titer considered by some as definite evidence of typhoid fever may occur in cases in which this infection is quite definitely excluded. They performed their tests with living cultures and usually also with formalized suspensions of *Bacillus typhosus*. Schilling and his associates have also reported upon a case of undulant fever in which agglutinins for *Bacillus typhosus* as well as for *Brucella* were present in the blood serum. Simpson reports that the Widal reaction was positive in low titer in only 2 of his cases of undulant fever and these individuals had previously been inoculated with a triple typhoid vaccine. He adds that for  $\frac{1}{2}$  years it has been their practice to test for anti-abortus agglutinins in all cases in which a negative Widal test has been rendered with the result that 12 cases of undulant fever were discovered in this manner.

Francis and Evans working with 100 sera of human cases of tularaemia found 37 that showed cross agglutination for *Brucella melitensis* and *Brucella abortus* as well as for *Bacterium tularensis* and that in 3 instances the agglutination titer was the same for the 3 organisms. In like manner 3 of 8 undulant fever sera cross agglutinated *Bacterium tularensis* but the agglutination titer was low. They however were able to differentiate the *Brucella* and *Tularensis* infections by the agglutinin absorption test and advise that the sera of patients suspected of undulant fever or tularaemia should be tested for agglutinins of both organisms unless the clinical history definitely points out the source of infection. If the difference in serum titer is marked the diagnosis is determined usually by the higher titer.

Nevertheless it is obvious that sometimes from the agglutination test the clinician may be puzzled as to whether to diagnose a case as one of undulant fever or of tularaemia. Even when the reaction occurs in some what higher dilutions of the serum with one of these organisms (*Brucella melitensis* for example) than the other (*Bacterium tularensis*) one cannot necessarily conclude that the former organism clumped in the higher dilutions is the one responsible for the infection. There is the possibility in question that this organism may be more spontaneously agglutinable than the other or more susceptible to the action of the nonspecific agglutinating substance present. While such serum may agglutinate both of these organisms it should be recalled that *Brucella melitensis* and *Bacterium tularensis* are very different microorganisms and have no etiological relationship. The diseases and pathological conditions they produce are entirely different. *Bacterium tularensis* is much more closely allied to *Bacillus peptis* than to *Brucella*. The writer has knowledge of a case that

was first diagnosed as undulant fever and subsequently as tularaemia by the agglutination test and finally typhus fever by the serum protection test. Simpson states that he found 6 instances in which the blood serum showed cross agglutination of *Brucella* and *Bacterium tularense*.

He however thought that the relatively high titer with the *tularense* antigen and typical clinical history of tularaemia left no doubt as to the diagnosis. Hegler reported 6 cases of undulant fever in which the diagnosis was made by the agglutination test alone. Two of the cases were fatal and attempts to cultivate *Brucella* at the autopsy of each were negative. Although the autopsies revealed pathological lesions evidently not associated at all with undulant fever and which would readily account for the symptoms and the death of the individuals the cases were diagnosed as undulant fever from the positive agglutination test alone. Weigmann who found that 219 sera agglutinated *Brucella abortus* also made cultures from 72 of the patients but in only 5 instances was *Brucella* recovered even though an atmosphere of 10 per cent CO<sub>2</sub> was maintained during incubation of the cultures. In 4 patients giving a positive serum reaction the fever appeared to be due either to typhoid infection or to croupous pneumonia. In 2 other patients a mixed infection was present. Five persons were detected on farms who gave a positive serum reaction without any history of illness. These however he considered were probably suffering from a latent infection.

It would appear probable that a number of febrile cases have been incorrectly diagnosed as undulant fever on account of the fact that the serum has contained either nonspecific agglutinins for *Brucella abortus* or *melitensis* or other substances have been present which have given rise to conditions simulating agglutination. Hence the clinician should bear in mind particularly when the diagnosis is made from the agglutination test alone that there are these sources of error.

Gibbes after pointing out that the great majority of the cases of undulant fever in the United States and about all of those from South Carolina have been diagnosed on the basis of the agglutination test collected the blood of 100 consecutive patients all of them afebrile at the time and free of all signs and symptoms that might be related to undulant fever and had the agglutination test for *Brucella abortus* performed at two widely separated laboratories. From this series he obtained reports of positive agglutination in titers of 1:100 or higher in 17 of the cases. In only one specimen of blood was there the slightest agreement between the two laboratories.

This was apparently from a case of undulant fever and upon which Laboratory A reported a positive agglutination in 1:1280 and Laboratory B a positive test in dilutions as high as 1:800. A specimen of this blood was then sent to a third reliable laboratory and the report was received that the serum failed to agglutinate any of the 11 strains of *Brucella* there in any dilution. In the other reports the two laboratories entirely disagreed that is all of the 35 sera that were reported as positive by Laboratory A were reported as entirely negative by Laboratory B and 4 that were reported as positive by Laboratory B were negative in Laboratory A. Four of the 100 patients had fever. One was regarded as a proved case of undulant fever on the basis of the clinical course of the disease and the positive blood culture. This patient as already noted gave a positive agglutination test in 2 of the 3 laboratories. The second patient ran a typhoid fever like course for 3 weeks and recovered before a diagnosis was made. His blood gave a negative test in Laboratory B and a positive test in a dilution of 1:100 in Laboratory A. The third febrile case had active autumnal malarial parasites in the blood and the fever readily responded to quinine. This

serum was reported negative by Laboratory B and positive by Laboratory A in a dilution of 1:100. The fourth febrile case had a post-influenzal respiratory infection with the fever disappearing as the signs in the lungs cleared. The serum was reported negative in Laboratory B and positive in Laboratory A in a dilution of 1:20. Of the 6 patients who were reported as showing agglutination reactions in dilutions of 1:400 none had fever and none of them gave a history of a typhoid-like illness in the past. Of the 3 patients who showed positive tests in dilutions as high as 1:200 only one had suffered in the past from an illness that had been thought to be typhoid fever. Likewise in the 11 patients who gave positive reactions in dilutions of 1:100 there was only one who had a history suggestive of typhoid fever. As has been pointed out some observers would regard these agglutination tests as evidences of a late or past infection in which no definite symptoms of disease were produced.

Recently Carpenter and Boak have emphasized caution in the diagnosis of the disease and they point out that the agglutination of the *abortus* antigen by the patient's serum is not always a safe criterion from which to draw conclusions and that a positive blood culture gives the most reliable information. Awe and Palmer also believe that the positive blood culture is the only accurate method of diagnosing the disease.

Huddleson also points out that the interpretation of a positive agglutination test in human blood in regard to active infection is often not an easy matter and that many individuals may have agglutinins in their blood from a past infection or from recent exposure. He believes this is especially true in veterinarians, packing house workers and breeders of livestock and agrees with the view that strains of *Brucella melitensis* tend to dissociate into antigenic variants and in this state are unreliable for use as antigens as they agglutinate nonspecifically. He thinks one should rely as much on the history of the case and other confirmatory tests as on the agglutination test in detecting active *Brucella* infection in human beings.

**Complement Fixation Test**—Apparently no distinct advantage has been reported for the complement fixation test over the agglutination test in the diagnosis of undulant fever though Williams and Kolner and Carpenter and Boak have recently employed it. Carpenter and Boak point out that it is more complicated and that often serums are found to be anticomplementary but still satisfactory as regards the agglutination test.

The technic is identical with that employed for the standard Wassermann test except that an *abortus* antigen is used. King reported to Carpenter that in many serums he was able to obtain a complement fixation reaction before he could demonstrate the presence of agglutinins in the serum. Larson and Sedgwick in employing the complement fixation test found that 17 per cent of the serums from 425 children with anatomical lesions of the osseous system (tuberculosis, rickets, etc.) contained *Brucella abortus* antibodies. They assume that the children were exposed to the infection because of the ubiquity of *Brucella abortus* in milk. Kristensen has also employed complement fixation particularly in checking his agglutination results. The results of the tests were not strictly parallel. Some sera which were strongly positive by the agglutination test were negative by the complement fixation test and vice versa. Of 277 sera which gave no agglutination only 2 gave a positive complement fixation reaction.

**Intradermal Test**—Meyer and Fleischner in 1918 demonstrated that infection of guinea pigs with *Brucella abortus* always produced cutaneous hypersensitiveness and that a positive intradermal test was a reliable index

of infection in these animals. The characteristic of a positive reaction was a marked induration, followed frequently by central necrosis. The reaction persisted for over 48 hours.

In 1922, Burnet recommended the intradermal test or cutaneous reaction for the diagnosis of undulant fever in man.

In his experiments on goats out of 645 tested 85 or 13 per cent reacted to the intradermal test while only 37 or 5.7 per cent gave positive serum reactions. When divided into 3 groups, 25 per cent of the goats gave both reactions while 9.5 per cent reacted to the intradermal but not to the serum test and 3 per cent gave agglutination but not the intradermal reaction. In performing the test on human beings he pointed out that while the agglutination reaction might be negative in from 15 to 20 per cent of the cases of undulant fever, in the majority of these cases the intradermal test gave positive results. The test was performed by the injection of 0.05 to 0.1 cc of a broth filtrate of a 20-day bouillon culture of *Brucella melitensis* (melitine) or *Brucella abortus* (abortine). In positive cases the symptoms begin from 6 to 10 hours after the injection in which there is a local reaction consisting of a slightly raised oedematous plaque 4 to 6 cm in diameter and distinguished by its red color from the surrounding skin. The reaction may be accompanied by pain and the local reaction may persist for 12 days. Burnet obtained positive results in cases of undulant fever from the eighth day onward and came to believe that it is of greater diagnostic value than the agglutination test.

Trenti also employed the filtrate of the bouillon cultures of *Brucella melitensis* for the test. On the other hand Mitra and Bua recommended killed cultures of *Brucella* suspended in salt solution for intradermal injections. Nattan Larnier who also used bouillon filtrates states that the intradermal method is more simple to perform and more constant in its result than the agglutination reaction or blood culture although in his hands it did not become positive until the seventh to the eleventh day of the fever.

Fornaciari using the intradermal test on 8 human cases found it positive in all in which the agglutination test and blood cultures were also positive. In 50 patients suffering from other diseases and 16 healthy subjects the test was negative. On the other hand Montagnani reported that the reactions produced by the filtrates are not specific as severe reactions may be produced in other morbid conditions and even in healthy persons of any age. He believes that an intradermal reaction if positive does not confirm a suspected diagnosis and if negative does not exclude undulant fever and thinks therefore that it is impossible to accept this reaction as of real practical value.

Bastai and Rotta employed filtrates of heat killed broth cultures in the intradermal reaction upon 19 patients suffering from undulant fever whose blood gave an agglutination in a dilution of 1:1000 and in one case 1:6000 and in which *Brucella* was isolated by haemoculture. As controls they tested the reaction on 72 healthy volunteers or on patients recovering from diseases other than undulant fever. Three of the latter gave a marked positive and 6 a weak positive the remaining 63 a negative reaction. Of the 3 positive cases the diagnosis of one was tetany and cholecystitis the second tuberculous adenitis and the third Dercum's disease. All of these also gave a positive tuberculin reaction. While Bastai and Rotta found the reaction constantly positive in cases of infection with *Brucella melitensis* they point out it is not strictly specific since it is occasionally present in uninfected individuals.

Duffau has employed an emulsion of the 3 test strains for the intradermal test injecting 0.1 cc of the filtrate (melitine) into one arm and 0.1 cc of the killed emulsion into the other. The reaction was positive in all of the cases which gave a positive blood culture but a modified reaction was obtained in 3 cases of typhoid and in 2 normal people.

In the United States Giordano first used filtrates of broth cultures but found that the reactions were not of sufficient specific value. Later on however he employed heat killed salt suspensions of the cultures and obtained strong reactions in known cases and not in controls. He performed the test upon 25 undulant fever 75 tuberculous people and 20 normal people. The suspensions of *Brucella abortus* were made from recently

isolated strains grown on Huddleson's liver agar from 48 to 64 hours and graded at a density of 1:1000 USPH standard. Two tenths of 1 cc was injected intradermally. A positive reaction appeared as a rule in from 12 to 48 hours. A small boil formed at the site of the infection with some induration. These boils contained neither pus nor bacteria. A small scar with an area of discoloration may result and last for some months. The 25 cases of undulant fever all gave a positive reaction while of the 100 controls only one was positive. Simpson reports that in 23 of the Dayton patients whose serums agglutinated *Brucella abortus* in titers ranging from 1:40 to 1:2560 strongly positive skin tests were obtained in every instance while 36 patients suffering from a variety of other diseases gave entirely negative results. In his series there was injected intradermally 0.1 cc of a saline suspension of the heat killed *Brucella abortus* adjusted to the standard used in the preparation of *abortus* vaccine (two billion per cc). Levin has also employed the intradermal test with a *Brucella abortus* antigen containing the dry bacterial protein. Tests were made on 365 persons. Positive reactions were obtained in 27 cases. Of the positive reactions 15 had clinical symptoms of undulant fever and positive agglutination tests. One had characteristic symptomatology of the disease but a negative agglutination test. Six had positive agglutination tests more than 2 years previously and had been patients in a hospital where there was an outbreak of undulant fever. Four gave negative agglutination tests and no history of an undiagnosed illness. Two students who gave typical positive reactions were healthy and gave no history of previous illness. The test was performed upon 33 hospitalized children. Only one child gave a positive reaction and her agglutination test was negative. She also gave a negative tuberculin test. The clinical diagnosis was sinusitis and mastoiditis.

Some other investigators have also found that the intradermal test is not entirely specific and that a positive reaction is sometimes obtained in normal individuals or those suffering with other diseases. Postow and Menefee (1938) point out that the test may be negative throughout the course of the disease in cases diagnosed by the isolation of *Brucella*.

**Allergy Test**—Huddleson (1940) has made use of a protein nucleate fraction of *Brucella* as an allergic agent for detecting *Brucella* skin allergy in human beings. He has made studies of more than 20,000 individuals who were either normal or actively infected and believes that it is a highly satisfactory and specific agent for detecting *Brucella* allergy.

He terms this agent *brucellegon*. He has also emphasized the use of the opsonocytophagic test as a means of diagnosis of brucellosis in human beings. This test depending upon the fact that the neutrophilic leucocytes in the whole citrated blood of human beings who have recovered from brucellosis phagocytize *Brucella* in large numbers. On the other hand leucocytes in whole blood from actively infected cases showed a lower degree of phagocytic activity and the blood of those who had no past or present history of infection showed little if any phagocytosis. However he found that the results of this test in patients with *Melioidosis* or *Brucellosis* often could not be given the same interpretation as the results of the test of patients with *abortus* or *swine* *Brucellosis*. Sometimes blood from which *B. melitensis* could be cultivated showed the same phagocytic picture that is presented by blood taken from immune individuals. He also points out that in tularaemia the phagocytic activity of the blood is considerably increased for *Brucella*.

Keller and his associates (1936) believe that the intracutaneous test may be used to determine a state of allergy resulting from *Brucella* infection but that it gives no indication of the immunity status of the patient. To determine the immunity status of individuals they state the opsonocytophagic test may be employed in conjunction with the intracutaneous test. The absence of marked phagocytic activity of the polymorphonuclear leucocytes in a patient with a positive skin test indicates infection and a lack of immunity. The presence of marked phagocytic activity would indicate either a developing or an established immunity. If marked phagocytic activity and a positive

skin test are demonstrated in a patient with fever they think it likely that the fever is due to some disease other than undulant fever. However some investigators do not place such reliance upon the opsonocytophagic test. Also both this test and the intradermal one have failed in a number of instances in which the diagnosis was definitely established by the cultivation of *Brucella*.

**Animal Inoculation**—Theobald Smith and Fabyan who first showed the susceptibility of the guinea pig to *Brucella abortus* infection pointed out it is possible to sometimes obtain pure cultures of *Brucella* from tissues contaminated with other microorganisms by inoculation of such material into this animal.

It has already been pointed out that the disease produced in the guinea pig is usually nonfatal and self limited. In some instances an infection somewhat resembling tuberculosis is produced characterized by the formation of small necrotic foci in the spleen liver lungs kidneys lymph nodes and epididymis and sometimes swelling of the carpal joints and ribs. The spleen and lymph nodes are usually particularly swollen. Microscopically there may be extensive proliferation of epithelioid and lymphatic cells followed by degeneration of other cellular elements. Gross lesions however are not always present in the guinea pigs as the recent experiments conducted by Hasley in the United States Wilson and Cruickshank and Barbour in Great Britain have shown. In such animals however the blood serum shows an agglutination test and the organism may be isolated from the spleen. Theobald Smith also pointed out that guinea pig inoculation is frequently of assistance in differentiating the bovine and porcine varieties of *Brucella abortus* the latter causing more severe lesions. Hardy found that the isolation of *Brucella* is often possible only through animal inoculation. The guinea pigs may be inoculated with the whole blood intraperitoneally or sediment from the urine or faeces injected subcutaneously in the groin. The organism may also be isolated from milk by injecting a 5 cc. of the naturally separated infected cream subcutaneously in each groin. An agglutination test should be performed on the guinea pig after 4 or 5 weeks with the serum separated from the blood and removed from the animal by intracardiac puncture. Animals showing a positive test are killed between the sixth and eighth weeks. The spleen liver and any enlarged lymph glands are removed and the cut surface of the organ is smeared on solid media for isolation of *Brucella* in culture.

**Blood Count.**—A few observers have suggested that the differential leucocyte count may be of assistance in making the diagnosis. However it should be borne in mind that the changes in the leucocytes in undulant fever are not peculiar to this infection alone. The leukopenia and lymphocytosis may in some instances be of assistance in excluding certain other infections particularly those where a leucocytosis is usually present with an increase in the polymorphonuclear leucocytes.

### DIFFERENTIAL DIAGNOSIS

Undulant fever has been confused with typhoid fever tularaemia tuberculosis visceral leishmaniasis aestivo autumnal malaria, liver abscess rheumatism influenza subacute bacterial endocarditis pyogenic septicaemia and in a few cases with appendicitis and cholecystitis.

Wise and Poston (1940) reported the co existence of *Brucella* infection and Hodgkins disease. They reported that they have cultivated in 14 consecutive cases *Brucella melitensis* from the blood or lymph, in all of which the diagnosis of Hodgkins disease has been made.

They raise the question as to whether the isolation of this organism from cases of Hodgkins disease represents merely latent *Brucella* infection or whether it may be of etiological significance. In attempts to obtain positive cultures of *Brucella* from

lymph nodes obtained from patients suffering with chronic disease involving the lymphatic system other than Hodgkins and residing in the same areas *Brucella* was isolated only once from cultures of 67 such lymph nodes. They believe that at least the *Brucella* infection particularly influences the syndrome of Hodgkins disease. They were able to induce disease leading to death in guinea pigs by intraperitoneal injections of blood or by a suspension of lymph nodes from patients with Hodgkins disease and to recover *Brucella* subsequently from the blood or tissues of these animals, but lesions identical with those of Hodgkins disease were not observed in animals. However it should be noted that the organisms they isolated from many of the patients suffering from Hodgkins disease possessed capsules and showed significant antigenic differences from the laboratory strains of *Brucella* infection.

**Typhoid and Paratyphoid Fever**—In undulant fever the obstinate constipation, severe sweating, relapses, fever and absence of rose spots may be points of differentiation from typhoid in which the more continued fever, the presence of rose spots, diarrhoea and tenderness in the right iliac fossa are more prominent symptoms. Nevertheless a few writers have reported the presence of occasional rose spots in undulant fever and diarrhoea has been occasionally met with in the more serious cases of this disease. Epistaxis and intestinal hæmorrhage may also occasionally occur in undulant fever and the sweating may be common to both of these diseases. The microscopical examination of the blood may not aid in the differentiation since the leukopenia and polymorphonuclear percentage reduction is somewhat similar in both. The lymphocytosis is often more marked in undulant fever. The agglutination test will sometimes give satisfactory and reliable differentiation but it should be borne in mind that the serum of undulant fever cases will sometimes agglutinate the typhoid bacillus. However the isolation of the specific organism in the respective diseases is decisive.

**Tularaemia.**—Owing to the fact that the blood serum of some cases of tularaemia may show an agglutination reaction for *Brucella* tularaemia may sometimes be confused with undulant fever. In tularaemia the history and often the presence of a local lesion or glandular enlargement may be of assistance in differentiation. The bacteriological examination should usually separate the two infections. However since *Brucella tularaemia* has not generally been detected or cultivated directly in the reported cases the investigator must sometimes rely for diagnosis upon an inoculation of the guinea pig with a suspension of the diseased tissue or a portion of one of the inflamed glands removed aseptically from the patient. Guinea pigs inoculated with such material containing *Bacterium tularaense* generally die after cutaneous or subcutaneous inoculation within a week. Post-mortem appearances are striking: the spleen is greatly enlarged and congested and both spleen and liver contain numerous necrotic foci in which *Bacterium tularaense* is present in large numbers. The pathological histology of the tularaemia infection is also distinctive in the guinea pig as first pointed out by Councilman and the writer (see page 79). If the material inoculated is from a patient with undulant fever either the death of the guinea pig does not occur or occurs only after several weeks or months.

**Tuberculosis.**—The evening rise of temperature with remissions in the morning in undulant fever and the persistence of these symptoms for a number of months even if cough is absent may very well cause the physician to consider the case as one of tuberculosis particularly when at the same time there is profuse sweating and loss of weight. In such instances the bacteriological procedures consist either of the isolation of a strain of *Brucella* or an undoubted agglutination test with it or the discovery of the tubercle bacillus or the only certain means of differentiating these infections. Roentgen ray examination of the lungs however may often give additional assistance. In military tuberculosis there is frequently an increase in the polymorphonuclear cells. In cases with meningeal involvement spinal puncture may reveal the tubercle bacillus.

**Leishmaniasis.**—The fever of kala-azar is frequently mistaken for that of undulant fever. It is a disease and both of these diseases have a number of other features in common such as emaciation, cachexia, tendency to hæmorrhage, anaemia, low polymorphonuclear count and splenomegaly. However in undulant fever the leucocyte count is rarely so low as it frequently is in leishmaniasis and in visceral leishmaniasis the spleen is often larger and harder and the liver more commonly enlarged. Splenic punc-



ture and cultures may in one instance reveal *Leishmania* or in the other *Brucella*. The agglutination test may also be of assistance in differentiating these infections.

In aestivo autumnal malaria the temperature, sweats and chills may be similar to those of undulant fever. The presence of malarial parasites in the blood and the cure of the infection by quinine in malaria should differentiate this disease.

Liver abscess in which septic fever, sweating and a tendency to anaemia are present may sometimes be confused with undulant fever. In liver abscess the history of previous dysentery may sometimes be obtained or amoebae demonstrated in the faeces or by liver puncture. There is very frequently a leucocytosis in liver abscess and the agglutination test if the case is undulant fever should give aid in differentiation.

**Rheumatism.**—In acute rheumatic fever swelling of the joints is more generalized, the inflammation often more acute and the cardiac complications much more frequent than in undulant fever. In undulant fever the salicylates appear to have little effect upon the process, endocarditis is comparatively rare and a leucocytosis is generally not present as it often is in rheumatic fever.

**Influenza.**—During the first few days of illness there might perhaps in some instances be confusion with influenza although the onset of influenza is usually more acute and sudden and the pulmonary symptoms are more marked than in undulant fever. The shorter course of influenza would also serve readily to differentiate this infection. Nevertheless Hardy says that about 20 per cent of the Iowa cases were erroneously diagnosed as influenza.

**Subacute Bacterial Endocarditis.**—This condition sometimes closely simulates undulant fever clinically. In a number of instances the diagnosis may depend entirely upon laboratory studies. In cases in which the pyogenic cocci are concerned a leucocytosis may be present. The isolation of the specific organism may be necessary for a diagnosis.

**Pyogenic Septicaemia.**—Frequently a focus of infection can be found. There is usually a polymorphonuclear leucocytosis and the affected joints often suppurate. Here again cultural studies and agglutination tests may be necessary to determine the nature of the infection.

**Appendicitis and Cholecystitis.**—Hardy and Simpson have called attention to the confusion of these infections with undulant fever. As noted Simpson has reported 12 appendectomies and 2 cholecystectomies which were performed on cases of undulant fever. In these the pathological examination revealed no evidence of an inflammatory process in the organs removed. The fever, abdominal pain and localized tenderness were the misleading features in these cases. Both the blood count and the bacteriological diagnosis should usually aid in differentiating disturbances such as these from undulant fever.

## PROGNOSIS

Hughes gives as the mean duration of the Caprine infection 70 days but points out that it may reach 300 days in some cases. Bassett Smith gave the duration as from 2 weeks to 2 years, the average in 522 cases being 4 months. In the United States Kern found that in 21 the disease lasted from 10 days to over 10 months but Hardy says that most of his patients found it difficult to tell just when recovery took place and as the onset was insidious he could not accurately determine the duration of the disease. In 212 of his cases the duration from the time the patient found difficulty in continuing his regular work until he was free from symptoms and able to resume it was as follows:

1 month or less 19 per cent 1 month to 10 weeks 7 per cent 3 to 4 months 34 per cent 5 to 6 months 11 per cent more than 6 months 9 per cent. The average total duration is therefore about 3 months. Simpson found the average duration of illness in 90 cases 4 months. He states that in one case there was convincing evidence

f relapses and remissions extending over a period of 8 years. DeBono (1940) has given the following analysis of 500 consecutive cases treated at the Central Civil Hospital Malta. Average duration of febrile stage: 1 month: 20 per cent of cases; 2 months: 25 per cent; 3 months: 4 per cent; more than 3 months: 15 per cent.

It is believed that infection with the bovine type is frequently milder than the porcine infections, with an average duration of 3 to 4 months; in about 20 per cent of the cases the patient being able to return to work within one month.

The disease is usually relatively mild in children, but the prognosis of the septicaemic cases is generally unfavorable.

The mortality of the European cases has usually varied from 5 to 6 per cent. Rozies has reported in Europe a mortality reaching 13 per cent. In the cases collected by Kern in the United States the mortality was 5.5 per cent, and in Hardy's series 3 per cent. Hence the prognosis in so far as continuance of life is concerned is good.

Nevertheless the frequent protracted course and prolonged invalidism reveal that the disease is more serious than the mortality indicates. Bassett-Smith emphasized that in Europe a most malignant condition may come on at any stage of the fever. We have already pointed out that death usually occurs from sudden hyperpyrexia, cardiac or pulmonary complications, exhaustion or haemorrhage. Bassett-Smith believed that a careful study of the relative quantity of the agglutinating substances in the blood will give some assistance in forming the prognosis. His conclusions stated briefly were: (1) with a persistent and high agglutination reaction the prognosis was generally favorable; (2) a steady rise from low to high agglutination indicates convalescence; (3) a rapid fall from high to low is unfavorable; (4) persistent presence of low agglutination titer is unfavorable. However, Manson-Bahr and Willoughby have since emphasized that the titer of agglutination appears to have no bearing on the prognosis, nor does it indicate in any way the probability of isolation of the organism by a blood culture. In the most severe cases which they encountered in their series the agglutination titer never rose above 80.

### PROPHYLAXIS

Since the great majority of the cases become infected from the consumption of milk or milk products, it is important that adequate protection should be given the milk consumer. It has already been pointed out that *Brucella* is killed by moist heat at a temperature of 60°C (140°F) in 10 minutes. The thermal death point fixed by Dalton and Evre for *Brucella melitensis* was 57.5°C. Zwick and Wedeman found that *Brucella abortus* was killed in 10 to 15 minutes at 60°C (140°F) and in 5 to 10 minutes at 65°C (145°F). Park mixed cultures of different strains of *Brucella abortus* with those of *Brucella melitensis* and made a milk suspension containing 5,000 million bacteria per cc. The organisms were killed when exposed to 140°F for 10 minutes to 142°F for 7 minutes and to 145°F for 5 minutes. Carpenter and Boak, who found that while a number of strains of *Bacillus abortus* grown in milk varied somewhat in their thermal death point, stated that all the organisms were killed after 20 minutes at 140°F. Hardy also observed that no *Brucella* organisms were living after an exposure to temperatures of 144 to 145°F for 30 minutes followed by rapid cooling in the ice box. Only Arnold has

reported that *Brucella* may survive after an exposure to the temperature of commercial pasteurization. In this instance it would appear that all portions of the milk had not been heated to 145 F for 30 minutes.

Complete pasteurization of milk and dairy products at 145 for 30 minutes, carefully supervised is apparently the logical method of eliminating milk borne infection. Although the ingestion of *Brucella abortus* in milk usually does not give rise to undulant fever in man until we have more accurate knowledge of the relationship between the ingestion of brucella infected cows milk and the production of the disease in man *pasteurization of milk is the only reliable procedure to adopt*.

Since infection may also occur through abrasions in the skin even when very slight prophylaxis should also include prevention of human contact with virulent organisms. Proper precautions should be taken to protect all those handling live stock or carcasses likely to be infected. It is believed that contact infection in packing house workers particularly in those who kill the animals may be reduced by giving more attention to the care of minor knife wounds or cuts in the skin. Protection with rubber gloves has been suggested. The control of the disease in cattle and goats has been attempted by means of vaccination using both killed and living cultures. Most of the results with killed cultures have not been very encouraging and a number of investigators report them as ineffective. While living cultures of *Brucella abortus* may have some prophylactic value the procedure also may be dangerous as the animals thus infected sometimes become chronic carriers of the disease and excrete virulent organisms in the milk for long periods. Cruickshank and Barbour did not find the use of living vaccines of *Brucella abortus* harmful either to infected or healthy cows. In 1 healthy cows the inoculation of living *Brucella abortus* vaccine did not result in the infection of the animals or in excretion of the organisms in their milk. However Theobald Smith pointed out that sometimes vaccinal strains may enter the udder of the cow and continue to multiply in the ducts and acini and he isolated the organisms from the milk of vaccinated animals. He also pointed out that prolonged multiplication in the udder might cause a change in the organism favorable to invasion of the human subject since in the udder ducts *Brucella* come into association with a variety of other bacteria.

An attempt has been made in recent years in the United States to build up *Brucella* free herds of cattle in a manner somewhat analogous to that employed in building up tubercle free herds. All cattle have been tested either by the agglutination test or by the bacterial examination of the milk and the reactors are segregated and their milk is used only after it has been pasteurized. The non reactors among the herds are kept entirely apart from the reactors and no physical contact is permitted after the stock is tested and new animals are only introduced if free from infection. The agglutination test has generally been employed for diagnosis in the animals but the intradermal test and bacteriological examination of the milk have also sometimes been used. Nevertheless Mohler as Chief of the Bureau of Animal Industry U S Department of Agriculture pointed

out that infectious abortion is so wide spread and the milk of so many animals infected that the main dependence for protection against whatever danger there may be from *Brucella abortus* in milk must be placed in pasteurization

In Malta an effort has been made to examine systematically all milch goats and to destroy those found infected. The incidence of undulant fever in Malta has been considerably reduced by this procedure but as Ascoli and Sanfilippo have pointed out in certain districts where a high percentage of the goats were affected serious difficulties resulted when an attempt was made to kill all the animals and notwithstanding the hearty cooperation of the sanitary officials this line of attack had to be given up.

Taylor and his associates (1938) report that *melitensis* infection of both goats and sheep is self limited and that hence the application of isolation methods and quarantine in regard to its control in these animals would seem worthy of trial. Huddle on (1940) and his associates in a study of the natural course of brucella in 3 naturally infected herds of hogs in Michigan reported that it is for the most part a self limiting disease. Many animals found infected by the serum agglutination test became negative to the test within a 90-day period. He thought that segregation of the animals found positive to the test would rapidly place the disease under control. Thomsen (1934) has reported completely eliminating swine brucellosis from Denmark by the application of the serum agglutination test and slaughter of the reactors.

Prophylactic inoculation has also been attempted in man. In connection with human prophylactic vaccination it should be borne in mind that experimental animals such as the rabbit and guinea pig which have been given repeated injections of killed cultures of *Brucella* usually show a high agglutination in the blood but such inoculations fail to protect the animal against the subsequent injection of virulent living cultures.

Hardy has also been unable to immunize guinea pigs with killed *Brucella*. More recently Gwathkin has made numerous attempts to protect guinea pigs by vaccines of *Brucella abortus* either killed by heat or sterilized by filtration against subsequent oral or ocular infection with a living culture. However all these experiments were unsuccessful. In one as many as 30 doses of vaccine were administered but without any significant effect.

Birt and Lamb reported an attempt to immunize a man by several preliminary vaccinations of dead cultures of *Micrococcus melitensis*. Subsequently an inoculation of a small quantity of a living culture was given and this was followed by an attack of undulant fever which ran a characteristic course. Eyre attempted to immunize 51 hospital attendants in Malta. The results were not very conclusive although he believed that prophylactic inoculation has a distinct value. Nicolle and Conseil administered killed cultures of *Brucella melitensis* both orally and by subcutaneous inoculation. A group of 3 men was employed. Two were immunized by subcutaneous injections being given 2 inoculations at 7 day intervals. Fourteen days later all 3 were inoculated with a living

culture the 2 vaccinated suffered no ill effects, while the control developed fever on the eighteenth day. A second experiment was carried out with 3 other men. Two were given 100 milliards of the vaccine by mouth on 3 consecutive days and again on the fifteenth day. Neither digestive nor general symptoms followed. 15 days after the last dose they showed no agglutination reactions. They, with the control were then inoculated with a living suspension of *Brucella melitensis* subcutaneously. The 2 vaccinated men were immunized, but the control developed fever on the seventeenth day, and from his blood the organism was recovered. It is stated that the blood of the immunized men in both series never showed any agglutination reaction.

Burnet immunized 2 monkeys with *Brucella abortus* one with a living culture and the other with a killed culture. Subsequent inoculation with living cultures of *Brucella melitensis* produced no effect. He also inoculated 3 men under the skin with doses of 200 million of *Brucella abortus*. They did not develop any fever and had no symptoms and were resistant to subsequent inoculations of *Brucella melitensis* whereas a control volunteer contracted undulant fever when inoculated with *Brucella melitensis*.

Dubois and Sollier during recent years, have again recommended the vaccination of human beings with killed cultures. Of 111 persons vaccinated all of whom were directly exposed to infection, there were no cases subsequently of undulant fever. On the other hand among 38 persons who were not vaccinated about 75 per cent of whom had no direct contact with infected animals 2 cases of fever developed.

Vincent Zammit Ceruti and Ascoli and Sanfilippo believe that they have been able to immunize goats by vaccination with large amounts of *Brucella*. Ascoli and Sanfilippo emphasize that it is necessary to use very large amounts of culture for immunization—from 16 to 20 plate cultures. Some goats inoculated with 6 plate cultures were not rendered immune. Other observers were unable to protect goats satisfactorily by vaccination.

Hence prophylactic inoculation is evidently still in the experimental stage. As yet the evidence is not entirely convincing that a satisfactory practical protection can be obtained by vaccination in either man or animals.

Disinfection of the urine and stools of patients should be carefully carried out and attempts may be made by the use of dyes (methyl violet and thionin) to eliminate *Brucella* which persists in the stools during or after convalescence.

## TREATMENT

Careful symptomatic treatment and nursing are most important in a disease which is often prolonged and unfortunately no entirely satisfactory specific treatment has been discovered. A trained nurse is particularly desirable and almost essential for many of the cases demand constant care. The high pyrexia, insomnia, delirium and other nervous as well as the circulatory, arthritic and gastro intestinal disturbances are usually symptoms requiring attention. The resourcefulness of the physician as well as the disposition and strength of the patient are often sorely tried in the

protracted cases. General therapeutic indications are rest in bed, maintenance of the patient's strength and nutrition and alleviation of the painful and distressing symptoms as they arise. In general the measures applicable to the treatment of typhoid fever are suitable to undulant fever. The pyrexia is best controlled by hydrotherapy and when sufficiently severe (103.5 to 104 F) cold sponge bath or ice pack should be given. Moderate degrees of fever (103 F or under) may be treated by tepid sponging. Bassett Smith emphasized that care must be taken never to check the sweats suddenly by hydrotherapy. Owing to the action of the toxin upon the circulatory system tachycardia and intermittency of the pulse may occur. Hence attention should be given to the pulse particularly in connection with the antipyretic treatment. If the pulse is sufficiently weak a stimulant may be indicated. While early in the disease stimulants are not usually required and should not generally be employed after the third or fourth week of fever and especially later they may at times when indicated be valuable and most useful in sustaining the more or less enfeebled circulation.

During the periods of fever the patient of course should remain in bed and in a disease of this nature which is often protracted in its course it is particularly important that the sick room should be sunny, well ventilated with an even temperature and with a bed that is comfortable. The patient should be kept in bed continually during the acute stages and until the temperature has been normal for at least 10 days and the tongue has become clean. Many relapses occur in patients who have been allowed to get up too soon. On account of the profuse sweating it is important that the bed clothing and linen should be frequently changed. The mattress should also be protected by a rubber sheet. Light woolen bed clothing has been recommended particularly on account of the fact that it absorbs perspiration more readily and prevents the patient from becoming chilled. The nurse should exercise care in keeping the skin in good condition. In the later stages of the disease patients are liable to develop boils and abscesses and precaution for the prevention of bed sores must be taken. Slight friction of the skin particularly when the patient is being dried after hydrotherapy as well as massage of any wasted muscle should be employed.

Constipation is generally a marked feature of the disease and should be treated with mild laxatives such as cascara sagrada, liquid petrolatum or petrol agar supplemented by enemata if necessary. During periods of fever a bed pan should be used.

For the painful arthritis hot applications are useful and opium fomentations or belladonna linament with rest and fixation often give great relief. Radiant heat and light therapy may also be employed. Frequently the insomnia is a distressing symptom and may require treatment with mild hypnotics. Headache is often relieved by the use of an ice cap. The nervous irritability may be benefited by bromides. Morphine is rarely indicated except when there is painful neuritis and when other measures including the administration of hypnotics have failed to alleviate

this For the treatment of the neuralgias or neuritis various forms of physiotherapy or electrical treatment in addition may be employed The anaemia should be treated by suitable diet and by the administration of iron in the form of Bland's pills or by the intramuscular injections of iron or arsenic In the advanced stages of the disease massage of any wasted muscles should be given daily Castellani recommends for severe vomiting which he observed in European cases sips of cold soda water or of champagne If the vomiting is persistent he recommends 2 drops of tincture of iodine in 1 ounce of cinnamon water or if this fails minute doses of cocaine gr  $\frac{1}{16}$  in chloroform water and a mustard leaf applied to the pit of the stomach

The diet is important and the appearance of the tongue is usually a good guide to the patient's digestive powers In the acute febrile stages the diet should generally be liquid With a fairly clean tongue and moderate fever a liberal diet can frequently be partaken of In the chronic cases with anaemia and impaired digestive function the maintenance of nutrition often becomes a difficult problem In general the patient should be given as much food as he can assimilate and in the later stages of the disease whiskey brandy and champagne are often very useful Alcohol is particularly valuable in undulant fever, as in typhoid fever since it acts as a food Warren Coleman points out that it is oxidized with the liberation of energy and spares equivalent amounts of carbohydrates and fat yielding 7 calories per gram It is in addition a circulatory stimulant as well In more critical stages of the disease, strychnine or caffeine may be employed as stimulants

In convalescence gentle exercise in the fresh air and sun are advisable If the disease has occurred in a tropical or subtropical region a change to a more bracing climate often results favorably

Disinfection of all contaminated material from the patient particularly the stools faeces and urine should be carried out The physician and nurse should bear in mind that the disease is infectious and the same precautions should be taken to prevent the spread of it as in typhoid fever for example

**Chemotherapy**—No drug has as yet been found which can be said to be a specific in the treatment of the disease Quinine salicylic acid and neosalvarsan have been tried but without favorable results

A few reports of the favorable use of mercurochrome have also been made However Ross and Martin in studying the effect of mercurochrome *in vitro* on *Brucella melitensis* and *Brucella abortus* found that in the concentration which is possible to attain in the blood stream mercurochrome has no destructive effect on these microorganisms

They treated 9 human cases with a 1 per cent solution Amounts up to 25 cc were given and doses from 2 cc to 15 cc Three of the 9 showed some improvement but 6 were not helped They concluded that no very definite proof had been produced that mercurochrome is likely to be of great value in the treatment of the disease They believed it was not advisable to exceed a dose of 10 cc of a 1 per cent solution in adults Other observers have thought this dosage was inadequate Kern reports that mercurochrome was given intravenously to 6 patients in the United States He thought that

its effect seemed to have been curative in 3 quite doubtful in 2 while in one it failed completely. Simpson reports that mercuriochrome was used in 6 of his cases without any appreciable effect on the course of the disease.

Acriflavine has also been employed in a few cases particularly in Italy. In Europe Izar treated 51 cases and found that the best effect was obtained if the drug was given in the maximum intravenous dose of 0.02 gm. for each kilogram of body weight or 0.6 for a man of 60 kilograms.

This dose, he says, may require being repeated once or perhaps twice and care must be taken that the drug goes directly into the vein. Hoffman believes that the drug has a favorable influence on the course of the disease. Thurber has reported upon 7 cases of undulant fever treated by intravenous injection of acriflavine. In 5 cases the fever was arrested within one month after starting treatment. In 2 the fever continued but at a low level. In 7 untreated cases the duration of the disease was from 9 months to 2 years, 2 cases ending in death. Thurber found that the best results were obtained in patients treated during the earlier weeks of the disease. If given after the development of arthritis acriflavine seemed to have little or no effect on this condition. He cautions that the injections must be given slowly to avoid reactions. Following them there occurs a 'cure', a rise and a final decline in the fever. No severe reactions were seen. He believes that by the use of acriflavine intravenously the course of the undulant fever can be materially shortened and the development of an incapacitating arthritis prevented.

Huddleson suggests that acriflavine seems to give the best results in relatively early cases and during a pyrexial wave when the temperature is still fairly high but provided there is no renal or hepatic impairment.

The dose employed was 10-15 cc. of a 2 per cent solution every second or third day for 3 or 4 doses. Temperature was said to fall with the first or second injection and remain at a subnormal level. The results, however, were not always so favorable and sometimes no impression at all was made or the temperature rose again. The treatment appeared to have been successful in about 25 per cent of the 40 cases in which it was used. In one case toxic jaundice followed treatment by it. Simpson reports that acriflavine was tried in 5 of his cases without any appreciable effect on the course of the disease.

**Sulfanilamide**—During 1938 some 15 reports appeared in the literature regarding the treatment of undulant fever with sulfanilamide and its compounds. In the reported successful treatment of 14 cases only 7 showed the disappearance of the temperature within 7 days after the beginning of the treatment. The fever disappeared usually in from 5 to 6 days after beginning treatment but in 1 case did not disappear for 44 days. The diagnosis was made by the agglutination test, except in 3 instances when *Brucella* was isolated from the blood.

In a case treated by Bartels (1938) on the first day 90 gr. of sulfanilamide was given and for 9 subsequent days 60 gr. a day. After 10 days the temperature was normal. On the other hand Kleeberg (1939) treated 6 cases with sulfanilamide but was unable to observe any influence upon the clinical picture. However it seemed to influence to some extent the bacteremia as was demonstrated by the examination of the blood.

Debono (1939) treated 25 cases in Malta with sulfanilamide. In all cases the diagnosis was confirmed by the agglutination reaction of over 1:100 of *Br. melitensis*. In 12 blood culture was positive. Sixteen were



of the ordinary type, 4 of the malignant and 6 of the mild intermittent type. In 18 prontosil rubrum was used in 4 prontosil album and in 3 streptocide. The average dose was 4.5 gm daily for 7 days but this was prolonged to 12 days in 4 instances and stopped earlier on account of intolerance in 5. In 19 cases there was no apparent effect of the temperature or on the course of the disease. In the other 6, 2 relapsed, one died, one was a very mild case and 2 recovered in the 8th and 17th week respectively. In 5 instances blood culture was attempted upon the seventh day of the treatment and in 4 of these it was positive. In the fifth the culture was contaminated. Debono is therefore definitely of the opinion that these drugs are not useful in undulant fever in Malta and in view of the usually low mortality of the disease and the definite element of danger in sulfanilamide therapy considers its use is not justified.

Bynum (1939) has reported 6 cases: 2 with acute, 1 with subacute and 3 with chronic brucellosis. The diagnosis was confirmed either by agglutination or skin tests. Sulfanilamide was given in adequate doses but although improvement followed in 3 later relapses occurred. Two of the patients experienced no relief of symptoms. Bynum emphasizes that he has not been able to obtain the satisfactory results reported by other investigators.

Horn (1940) has reviewed the treatment of 83 cases by different observers with sulfanilamide or its derivatives. He found that the reports indicate a favorable influence on the subjective symptoms of the disease in a ratio of 2 to 1. He elicited this same ratio in 54 cases. However only 2 of the cases were acute and in one of these sulfanilamide was regarded as resulting in a cure with 40 grains (2.6 gm) daily for 17 days while the other patient failed to respond and died 8 months later although she was given 3 additional courses of the drug.

Huddleson (1940) also points out that while the early reports indicate that it might be a specific in a large number of acute and chronic cases it has had no effect on the course of the disease. He observed its use in 15 patients and in none of these was the course of the disease affected. Spink (1940) also reports disappointing results in several patients with undulant fever treated with sulfanilamide.

*Vaccine therapy* has been employed for many years in Europe and for more than 10 years in the United States. Kennedy, Eyre, Castellani, Bassett Smith, Owen and Newham, Gruffire and Prausnitz have among others recommended the use of vaccines in certain cases. In earlier years Eyre thought prophylactic inoculations had distinct value while Castellani reported that he found vaccines useful occasionally in protracted cases with very low fever. Bassett Smith after many years experience in treatment thought that vaccines might be used with good results particularly in subacute and chronic cases. From a large series of cases he believed that their use was of no advantage in the acute stages of the disease. He pointed out that methods which increase the phagocytic activity of the white cells in the blood such as the administration of yeast nucleic acid combined with appropriate doses of vaccine are likely to be most beneficial.

Manson Bahr and Willoughby tried many varieties of vaccine in 6 cases with large doses. They obtained no evidences that the use of vaccine given over a prolonged period of 3 months or more in any way modified the course of the disease. However Manson Bahr (1936) points out that while stock vaccines are of little benefit the use of an autogenous vaccine prepared with the infective organism of the patient in chronic cases sometimes results in a lowering of the temperature and the clinical improvement of the patient's condition. Protein shock therapy with nonspecific protein sometimes leads to the same result after producing a febrile reaction with rigors.

Simpson (1930) in 46 cases employed a vaccine made from heat killed *Brucella abortus* standardized to 2 billion per cc. with such apparently favorable results that he decided to employ it as a routine treatment. The vaccine was given by deep subcutaneous injection. The usual dosage was 0.25 cc. for 3 injections followed by 0.5 cc. for 3 injections followed by 1 cc. doses all at three day intervals. The first one or two injections were followed by a mild or moderately severe general reaction in two thirds of the cases following which the reaction diminished in intensity after each succeeding vaccination. In several instances the site of injection remained indurated for many days but no necrosis or abscess developed. Following the first two or three injections the fever usually approached a normal level and the symptoms abated. As a general rule those patients who experienced the most marked general reaction had the most rapid favorable response to the vaccine.

In order to determine whether or not the results obtained were entirely due to a foreign protein Simpson employed typhoid vaccine in 8 cases and sterile milk was used in 4 instances. In these cases there was a much more marked elevation of temperature following the injections but the subsequent course of the disease was not appreciably altered. He points out that due caution must be exercised in the valuation of any therapeutic measure in a disease characterized by natural remissions.

Hardy reports that while vaccination has not been used in a large number of the Iowa cases they have observed rapid recovery following administration of the vaccine but have also seen other cases whose infections continued unmodified by the same treatment.

Huddleson also emphasizes that many cases have not been benefited by its use and there is still very great difference of opinion in regard to its value.

Ramsford (1935) who has employed it extensively in the treatment of the disease in Malta believes that if recovery is not obtained after 2 or 3 injections of the vaccine its continued use is likely to result in more harm than benefit to the patients.

Huddleson and Johnson (1939) have recommended a (Seitz) filtrate of a culture of *Brucella* grown upon liver broth for intramuscular injection to which preparation the name of brucellin has been given. If precautions are taken to determine the sensitiveness of the patient before administration Huddleson believes that it can be used without dangerous

of the ordinary type, 4 of the malignant and 6 of the mild intermittent type. In 18 prontosil rubrum was used in 4 prontosil album and in 3, streptocide. The average dose was 4.5 gm daily for 7 days, but this was prolonged to 12 days in 4 instances and stopped earlier on account of intolerance in 5. In 19 cases there was no apparent effect of the temperature or on the course of the disease. In the other 6, 2 relapsed, one died, one was a very mild case, and 2 recovered in the 8th and 17th week respectively. In 5 instances blood culture was attempted upon the seventh day of the treatment and in 4 of these it was positive. In the fifth the culture was contaminated. Debono is therefore definitely of the opinion that these drugs are not useful in undulant fever in Malta and in view of the usually low mortality of the disease and the definite element of danger in sulfanilamide therapy considers its use is not justified.

Bynum (1939) has reported 6 cases, 2 with acute, 1 with subacute and 3 with chronic brucellosis. The diagnosis was confirmed either by agglutination or skin tests. Sulfanilamide was given in adequate doses but although improvement followed in 3 later relapses occurred. Two of the patients experienced no relief of symptoms. Bynum emphasizes that he has not been able to obtain the satisfactory results reported by other investigators.

Horn (1940) has reviewed the treatment of 83 cases by different observers with sulfanilamide or its derivatives. He found that the reports indicate a favorable influence on the subjective symptoms of the disease in a ratio of 1 to 1. He elicited this same ratio in 54 cases. However only 2 of the cases were acute and in one of these sulfanilamide was regarded as resulting in a cure with 40 grains, 6 gm daily for 17 days while the other patient failed to respond and died 8 months later although she was given 3 additional courses of the drug.

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febrile periods and afebrile lapses and sufficient evidence is not yet available to determine the definite efficacy of any of these means of treatment

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consequences. The dose is gauged by means of a preliminary intradermal injection of 0.2 cc. If the local reaction is very severe and there is no immediate fall in temperature the treatment is abandoned as too dangerous.

If there is no reaction at all as happens in a number of early cases the treatment is postponed until an allergic response is developed. If the reaction is moderate a dose of between 0.5 and 1 cc. is injected intramuscularly. After 3 days a slightly smaller dose is injected. If decided improvement is not obtained by the fifth injection it is felt that it is useless to proceed. It is not advised to give an inoculation with brucellin if the temperature is above 103. Huddleson believes that brucellin favorably affects the course of the disease by producing a systemic allergic reaction which is accompanied by a neutrophilic polymorphonuclear leucocytosis and increase in immune opsonins.

He reports that in more than 500 cases treated with brucellin the disease was favorably influenced in approximately 85 per cent. Nevertheless brucellin fails to affect the course of the disease in approximately 15 per cent of the cases treated. He points out that it is difficult to evaluate the efficacy of specific treatment since it is a well known fact that the symptoms in many cases are only of short duration. A total of 86 patients were studied at Malta. 28 remained untreated as controls and 12 of these recovered in 20 days. The remaining 16 showed symptoms of the disease 2 months after admission. During the duration of the disease treatments in 20 of the cases varied from 2 weeks to 1 year. The injection of brucellin had little if any effect on the course of the disease in 7 cases. The average duration of symptoms in the remaining 51 cases after beginning treatment was 12 days, the shortest being 3 days.

Earlier reports upon the value of *serum treatment* of the disease have not been encouraging. Foshay and Wherry (1935) have employed a detoxified bacterial antigen in the preparation of an antiserum for the treatment of undulant fever. Goats were used at first in the preparation of this antiserum but more recently horse serum was found to be equally satisfactory. Flippin (1938) has reported encouraging results in the treatment of 5 cases with a polyvalent anti melitensis serum of bovine origin. Creswell and Wallace (1936) have reported remarkable results from the injection of patients with whole blood from individuals who have recovered from the disease. They used the phagocytic test as an index in the selection of appropriate immune donors and reported that the phagocytic index ran parallel with the clinical condition of the patient, being low during clinical manifestations of the disease and high following recovery.

Non specific protein therapy has also been employed in treatment and several clinicians (Erwin Hunt and Niles 1936) have inoculated typhoid and paratyphoid vaccines intravenously with reported success. Simpson, however found that the subsequent course of the disease was not appreciably altered by such treatment.

Prichman and his associates (1938) have advocated physically induced hyperpyrexia as valuable.

Obviously a decision as to the value of any method of treatment in undulant fever is difficult especially on account of the alternations of

## Chapter XXII

# LEPROSY

### DEFINITION AND SYNONYMS

Synonyms—*Lepra* elephantiasis graecorum leontiasis satyrnasis  
French *La lèpre* German *Aussatz* Norwegian *Spedalskhed*

Definition—Leprosy is an infectious disease peculiar to man with a prolonged incubation period and chronic course. It shows itself in most cases by pigmentary changes in the skin and by the formation of characteristic nodules particularly in the skin mucous membranes nerves bones and viscera which give rise in some instances to thickenings and granulomatous tumors and in others to alterations in sensation (analgesia) anaesthesia and to degeneration of tissue ulcerations progressive constrictions and mutilations of the extremities. It is caused by *Mycobacterium leprae* which is especially prevalent in the granulomatous lesions. The infection after a long course is usually fatal.

There are two well recognized types of the disease (1) neural and (2) nodular. The type characterized by granulomatous proliferations in the corium and subcutaneous tissues as well as the lymphatic glands is known as nodular or skin leprosy. It shows spots and nodular infiltrations chiefly about the lobes of the ears alae of nose and region of the eyebrows with falling out of hairs of the eyebrows and the bearded region. It also involves the extensor surfaces of the forearms and dorsal surfaces of the hands and feet. The palms of the hands and soles of the feet are almost never invaded. The other type is known as nerve or maculo-anesthetic leprosy and is characterized by nerve thickenings flat anaesthetic spots chiefly of the covered region of the body muscular palsies and atrophies with trophic changes leading to contractures and mutilations. When the two types are associated the condition has been designated as mixed or cutaneous leprosy. The majority of the cases are of the mixed type as it is rare to see a case in which the lesions are limited entirely to the skin.

The International Congress of Leprosy (1938) recommended (with reference to the earlier classification of leprosy into the neural and cutaneous types) that while the term neural should be retained because the term cutaneous leprosy had proved confusing its use should be discontinued and replaced by the term lepromatous. They defined the two types of the disease as follows

Neural (N) Type—All cases of the benign form of leprosy with disturbances of pyreneuritic nature (i.e. alterations of peripheral sensation trophic disturbances atrophies and paralyses and their sequelae) or macules of nonlepromatous nature

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The early history of the disease in Europe is confused particularly by the fact that leprosy was often confounded with elephantiasis or Grecian leprosy an entirely different disease or with syphilis and various other skin diseases. It has been suggested that the Greeks called the disease elephantiasis because of the stump foot and dragging gait so frequently present. There seems to be general agreement however that the endemic leprosy of Egypt was not known to the older Greek physicians practicing in Greece and that it did not show itself in the Roman Empire until the last century B.C. It appears that the disease was introduced into Europe from Egypt in the First Century by the returning legions of Pompey. Aretaeus in the Second Century A.D. gives a good description of it. It however was a still longer time before it attained a general diffusion in Europe.

For the history of leprosy in the countries nearer Asia our more definite information goes back to the ninth and tenth centuries of the Christian era. The writings of the Arab physician Rhazes and others of this period give definite knowledge of the prevalence of the disease in Mesopotamia Syria and Persia. In the western hemisphere Hirsch tells us according to the uniform statement of authorities in Guiana and the West Indies leprosy was unknown until the arrival of the negro slaves. In Bahia the earliest account of its occurrence goes no further back than 1755. Also in Paraguay and Uruguay the outbreak of the disease is traced to the introduction of the negro race.

During the Middle Ages leprosy increased enormously. In northern Europe it began to appear in the 6th and 7th Centuries and its spread with the crusaders was appalling reaching its full height in the Thirteenth Century. The leper wandering abroad an outcast from human society living apart in huts in the open fields became a common figure often referred to in the literature of the period.

The lepers at this period were compelled to wear a special dress to use a clapper when passing along the roads to indicate only with a stick the articles they desired to buy in a market while they were forbidden to drink from public fountains to touch children to speak to a healthy person in a loud voice or to eat with any person other than a leper. Further the church performed a burial service over a person who was diagnosed as a leper and therefore officially he was dead.

There are legislative enactments of historical importance against the marrying of lepers and on their segregation made by Rothar King of the Lombards in the Seventh Century by Pippin in 757 and by Charlemagne in 789 one for the empire of the Franks in the 8th Century and for England in the year 950. Lepers' hospitals for the care of these unfortunate were already mentioned by Gregory of Tours about 560. In the 8th Century there are references to the reconstruction in the Frankish kingdom and in the Ninth in Ireland. As the disease spread far and wide the advantage of these retreats for purposes of segregation became apparent and they turned out to be an important factor in the eventual stamping out of the disease. In England the first leper asylum was founded in Canterbury in 1096. From England the disease spread to Scotland Robert Bruce dying of it in 1309. Some idea of the importance which leprosy had reached during the Thirteenth Century may be obtained from a knowledge of the fact that during that century there were 10,000 of these lazarettuses or leprosia as they were called 2,000 of which were in France alone. Vichow in his study of leprosy in the Middle Ages has given an account of these institutions in Germany during the 13th and 14th centuries and has emphasized the importance which they played in the subsequent suppression of the disease. He points out that their erection represented not only a wave of human charity but a great social and hygienic prophylactic movement. Leprosy had disappeared as an epidemic by the middle of the 6th Century and as it diminished the number of leper houses began to decrease. The disease had so completely died out by the end of the 16th Century that in 1656 Louis XIV. was able to abolish the leper houses and devote their endowments to charity and to the construction of general hospitals. At this time it was found that many individuals suffering with other diseases resembling leprosy had also been con-



(i.e. leprides usually with localized sensory disturbances) or both. These cases give evidence of relative resistance to the infection are of relatively good prognosis as regards life although mutilation may take place and usually react positively to leprolin. Bacteriologically the skin lesions are typically but not invariably found negative by standard methods of examination though the nasal mucosa may be found positive. Many of these lesions are histologically of tuberculoid nature.

**Lepromatous (L) Type**—All cases of the malignant form of leprosy relatively nonresistant and of poor prognosis usually negative to leprolin exhibiting lepromatous lesions of the skin and of other organs especially the nerve trunks. Bacteriological examination usually reveals abundant bacilli. Disturbances of polyneuritic nature may or may not be present they are usually absent in the earlier stages and present in the later stages of primarily lepromatous cases and are often present in cases arising secondarily from the neural form.

The neural type of leprosy may be divided into two subtypes namely anaesthetic and macular. In the anaesthetic variety of neural leprosy there is evidence of involvement of the nerve trunks only (polyneuritic changes and sequelae without macular skin lesions) in the macular neural type the lesions may consist only of flat macules or in other instances of raised tuberculoid macular patches.

Leprosy is caused by an acid fast bacillus which has not been satisfactorily cultivated or inoculated into animals with successful reproduction of the human lesions. It is found in extraordinary abundance in the granulomatous subepithelial tissues of nodular leprosy. In nerve leprosy however it may only be present in scanty numbers or not found at all. It is especially likely to be found in the perineurium and endoneurium of the ulnar, facial or perineal nerves.

**History**—Historical records are not sufficiently definite to allow one to decide where leprosy originated but it is certainly of great antiquity. Thus leprosy is often referred to in the Bible but from some of the references to it it is evident that other skin diseases and particularly psoriasis were sometimes confounded with it as would obviously be natural at that period. However in Leviticus Chapters XIII and XIV truly remarkable passages regarding the diagnosis and prevention of leprosy are to be found. More or less definite directions are given regarding segregation and disinfection and the incineration of the patient's garments and the closing of the house in which he lived. These directions regarding leprosy continued in force in the Middle Ages. Notwithstanding this fact some authors have claimed that the biblical references are not sufficiently distinct for us to be sure that leprosy is referred to and that the word

Zaarith under which leprosy was perhaps designated had a theological rather than a medical meaning. Accounts that seem reliable of the occurrence of the disease on extra-European soil date from the time of the exodus of the Israelites from Egypt their wanderings in the desert and the establishment of their power in Palestine. It appears moreover that leprosy had been endemic in Egypt from the remotest times since in the Egyptian papyrus discovered by Brugsch in 1875 a disease resembling leprosy is mentioned as early as the reign of Hesepti of the First Dynasty probably 4600 years before the Christian era and more importantly it is also referred to in the Ebers papyrus compiled 1500 B.C. Leprosy seems to have been likewise prevalent in ancient days in Persia where before the time of Herodotus (Herod I) there were stringent laws for the expulsion of lepers from the towns. It was known as the Phoenician disease which suggests Asia as its origin.

It also appears to have been known in India during period of very great antiquity. In the Rig Veda Samita of Atreya about 1400 B.C. references to the disease are apparently given under the term *Kuhta* and from the writings of Charaka and Susruta we can apparently distinctly trace its occurrence in India to the 7th century B.C. In Japan it seems to have been recorded first in 1250 B.C. and in China one or two centuries before Christ.

Venezuela and Cuba. It is not uncommon in Central America. Cuba and the West Indies. The above figures relate only to typical advanced cases and obviously do not include many infected areas for which no data are available. In some regions the disease is on the increase.

In the *United States* McCoy (1938) reports that there are some 400 to 500 active or registered cases of leprosy and at least half as many not known (or registered). At the national leprosarium at Carville, La., there have been some 400 cases of leprosy. The majority of these came from Louisiana and Texas, a small number from Florida. A few cases have also originated in South Carolina.

In 1938 there were 365 cases, 75 discharges and 59 new admissions. In earlier years in Minnesota and the adjacent states there were some 200 cases, the great majority being Scandinavian immigrants. However, in recent years the disease has shown no tendency to spread in the Northwest and has practically disappeared in this center. A small focus existed for over 100 years at Tracadie, New Brunswick. During 1933 there were cases of leprosy diagnosed in Colorado, Illinois, California, Minnesota, New York, Texas, Maryland, North Dakota, Massachusetts, Nevada, Pennsylvania, Louisiana, Nebraska, New Jersey, and Arizona. In addition to the infection in these states, cases of leprosy were diagnosed in 1924 in Georgia, Kansas, and Salt Lake City. Fordyce (1935) reported upon 47 cases which came under his observation in New York City. Blumer has seen 5 cases in Connecticut in 19 years. So the physician may meet with sporadic cases from time to time scattered throughout the United States.

### ETIOLOGY

*Mycobacterium leprae* (*Bacillus leprae*) Hansen, 1874.—This organism is the accepted cause of human leprosy and is usually present in great profusion within characteristic cells in specific lesions. The constancy with which it is found in the lesions and the failure to find another cause make its acceptance as the etiological factor of leprosy almost universal. However, absolute scientific proof that *Mycobacterium leprae* is the sole etiologic agent still requires satisfactory cultivation and the reproduction of the infection by satisfactory inoculation of man or suitable animals.

**Morphology**—*Mycobacterium leprae*, as it occurs in typical lesions, exhibits marked polymorphism. Its form and structure are particularly revealed when tissue films are gently steamed with carbolfuchsin for 5 minutes, decolorized in 2 per cent hydrochloric acid, 0.5 per cent sulphuric acid, and counterstained with methylene blue. The bacilli vary in size from 1.5 to 5  $\mu$  in length and from 0.2 to 0.5  $\mu$  in width, with parallel edges and rounded ends. Curved forms with pointed ends are not uncommon. The organisms stain quite uniformly in preparations made from the tissues in the quiescent phase of the disease. In the reactionary and resolution phases or under the influence of treatment, the individual organisms may be multigraular, coccoid, monopolar, or bipolar. Round spore-like bodies which stain more intensely and have a diameter greater than the thickness of the cell, often give a distinct granular appearance to a clump of the bacilli. A so-called ultra-virus stage in the life cycle of the bacillus and in the organism of rat leprosy has been reported by several observers. Its existence might explain the rarity of acid-fast bacilli in the early lesions and in the more advanced lesions of the neural type. However, as yet there is no definite evidence that such a virus stage exists and no satisfactory susceptible animal is known with which to demonstrate the existence of such a virus stage in the human organism. Experiments with the inoculation of filtrates of the rat leprosy bacilli are inconclusive (Lowe, 1937).

fined in these institutions with true lepers. Relics of the disease in the art of this period are preserved in Rubens' painting of St. Martin in Windsor Castle sometimes attributed to Van Dyck and Munillo's St. Elizabeth in the Prado.

**Geographical Distribution**—Leprosy is more particularly a disease of tropical and subtropical countries, though it is rather widely diffused in many parts of the world. It has been estimated that there are some three million cases in the universe. In Europe during 10 years some 7000 cases have been reported with about 1000 each in South Russia, the Baltic Provinces and Crete, and some 500 each in Turkey, Rumania, Spain and Portugal. There are also other scattered cases in northern Europe, no part of which may be said to be entirely free from the disease.

In France during recent years a fairly large number of cases have been reported and the disease has been fairly prevalent in parts of Prussia. However in Europe in recent years the disease has shown no tendency to spread. Flandin and Ragu recently conducted a careful study of the origin and mode of contagion of 95 lepers in Paris and its environs and found that only 6 had contracted the disease in France. Macleod (1939) reports that there are a few lepers still in Great Britain but the majority are adults who have returned from tropical countries. In Italy the same thing is true. The cases are generally found in peasants who have returned after migrating to Latin America. However occasionally cases develop in Italy by contact in individuals who have not been away from the country. In Norway, Sweden, Iceland and Finland where the disease was formerly common it has become greatly reduced in recent years. In 1937 only some 80 cases were reported in all these four countries.

In Asia leprosy is widely distributed, it being estimated that there are some 1,250,000 cases scattered particularly through China, Japan and India. Indo-China, Siam, the Philippine Islands and the East Indies with smaller numbers reported in Malaya, Ceylon, Persia and elsewhere in the interior. In India it was estimated in 1893 that there were over 700,000 cases and in Japan in 1929 over 30,000 while the Island of Java has over 4000 cases. In the Philippine Islands Rodriguez (1938) reports the total number of lepers under segregation as 8,566. However in the survey conducted in the Island of Cebu it was found that for every leprosy case open (one with active lesions) one closed case was found, roughly speaking. Applying this ratio to the rest of the Philippines he believes there are approximately 10,000 cases of leprosy throughout the Islands as a whole. Although the disease is found in all the provinces of the Philippines the incidence varies greatly, varying from 0.8 per 10,000 for the mountain provinces to 16.3 per 1,000 for the Province of Cebu. In Hawaii several years ago it was estimated that there were about 700 lepers. Leprosy was introduced into that country about 1854 and reached its maximum prevalence in 1894, since which time there has been a slow decline, but still from 50 to 100 new cases are reported each year. In the Australian group of islands and in the islands of the Pacific leprosy is fairly common.

In Africa it is estimated that there are some 500,000 cases of leprosy. It is particularly prevalent in the southern part and is noticeably on the increase. The disease is found in most parts of tropical central Africa where it has been looked for and particularly besides in South Africa, in Egypt and Madagascar. Recently (1939) reports indicate that one of the highest endemic centers is probably in Central Africa where in parts of the Belgian Congo something like 10 per cent of the population was said to have leprosy. A decade ago at Panyang in the Cameroons it was reported that 25 per cent of the natives suffered from leprosy. Scott (1939) states that the territory extending across Central Africa from Nigeria to Abyssinia is the most severely infected in the world. However in 1935 in this country where 15 leprosy treatment colonies had been established the rate in the southern part of the country was reduced to 36.3 per mille.

In the western hemisphere it is estimated that there are some 30,000 cases. The disease prevails throughout South America, especially in Brazil, Guiana, Colombia

In recent years, the relationship of these cultures to the true leprosy bacillus has been questioned. While with some of the cultures transient granulomatous nodules have been produced in animals also similar nodules have been obtained with other acid fast bacteria such as the hay bacilli (*M. phlei*).

The employment of various serological tests for the determination as to which type of organism cultivated is the etiological factor in the disease has not led to any very definite results. Neither the study of the agglutination test nor the complement fixation one has been of assistance in this connection so that at present many investigators are not convinced of the successful cultivation of *Mycobacterium leprae*. Furthermore the inoculation of animals with human leprosy material containing enormous numbers of organisms does not give rise to typical progressive lesions and the question of the identity of any organism isolated must therefore remain open.

Soule and McKinley (1936) incubated leprosy suspensions in different media and especially in formal glycerin agar in varying partial presence of oxygen and carbon dioxide 40 and 50 per cent respectively and obtained 16 subcultures in 18 months in each case of a distinct bacilli. In 1937 McKinley and de Leon reported having carried the cultivation through 60 generations. Salle and Moser working in the Carville Leprosy Settlement and using glycerin veal agar with chick embryo placed under the gaseous conditions recommended by McKinley obtained a slow and non acid fast rods the former most numerous in young cultures and becoming fewer as the culture aged and more frequent on subculture. During 1936 at the Calcutta School of Tropical Medicine attempts were made to confirm this work. Twenty-four series of experiments were carried out and 1000 tubes of different media inoculated. Half were carefully incubated in the usual way and half under the gaseous tension recommended by McKinley and Soule. The tubes were examined periodically over several months. Of 70 tubes sealed in August 1936 and kept in a gaseous environment of 40 per cent oxygen and 10 per cent carbon dioxide 35 showed slight macroscopic and considerable microscopic evidence of colony formations and many masses of acid fast bacilli were seen in the smears. Of another 70 under ordinary atmospheric conditions 11 showed similar but less evident growth. Subcultures were being attempted but no multiplication of the bacilli had so far been proved nor had the organism been demonstrated to be *Mycobacterium leprae*. In minced chick tissue medium bacilli persisted for a long time but there was no multiplication. Low (1939) reports that while the work in cultivation at Calcutta has been continued no definite multiplication has been observed in cultivations of either the human or rat leprosy bacillus.

Eddy (193) has also attempted to cultivate *M. leprae*. She employed 63 different culture media and incubated the tubes (1) under aerobic conditions (2) under partial tension conditions (3) with increased carbon dioxide and oxygen as recommended by Soule and McKinley and (4) under anaerobic conditions. No growth of any microorganism that resembles *M. leprae* as it occurs in the tissues was obtained. However she did isolate a strain of the avian tubercle bacillus on such media.

Birkhaug (193) from Norway has also been unable to get an actual growth of the organism. Loving (1943) has reported the cultivation of the Hansen microorganism in vitro in the presence of a medium enriched with thiamine / vitamin B<sub>1</sub>.

The majority of the Committee of the International Leprosy Congress in 1938 agreed that the problems of the *in vitro* growth of the causative agent of leprosy have not yet been solved satisfactorily. Soule states that while his cultures are still alive after more than 60 transfers the growths are still very slight. McKinley (1938) stated that the limited

**Distinction from *Bacillus tuberculosis***—In size shape and staining reactions *M. leprae* at times is practically indistinguishable from the tubercle bacillus but usually can be differentiated by the following points

(1) Leprosy bacilli are found ordinarily in huge numbers in the lesions chiefly within the so called lepra cells and are often grouped in packets like a bundle of cigars tied together or arranged in a palisade. Chains are never seen. Most noticeable is the presence of both intracellular and extracellular globular masses known as globi which consist of clumps of bacteria enclosed in capsular material.

(2) Leprosy bacilli usually stain more solidly and the granules are coarser and more widely spaced. They may be stained by the ordinary strong bacterial stains such as cold dilute carbol fuchsin and by the Gram stain with which the leprosy bacillus is Gram positive. In a few instances Gram positive variants have been described.

(3) The leprosy bacillus does not resist the decolorization in acid fast staining quite so well as the tubercle bacillus although there is some variation in the individual strains in this respect. With the Ziehl Neelsen method with 3 per cent HCl in alcohol it decolorizes much faster than the tubercle bacillus while with 20 per cent H<sub>2</sub>SO<sub>4</sub> in water it may hold its color almost as well. Some prefer to use for decolorization a 5 per cent solution of the acid.

(4) The leprosy bacillus has not been satisfactorily cultivated and does not on inoculation produce disease in animals and it may be differentiated especially from the tubercle bacillus by its failure to cause characteristic lesions in inoculated guinea pigs.

**Cultivation** of the leprosy bacillus has been attempted repeatedly with for the most part, negative results. Some investigators however have obtained growths from leprosy lesions of acid fast bacilli (both chromogenic and non chromogenic) or of acid sensitive diphtheroids which in some instances developed acid fast forms upon cultivation. Branching bacilli and granular and coccoid forms have also been described. Recently several investigators have reported such growths from filtered extracts of leprosy nodules (both human and rat). It is believed by some that these various types (including a filtrable form) represent different phases in the life cycle of the leprosy bacillus which in the human body exists as an acid fast tissue parasite and multiplies only within the cells.

The organisms which have been particularly cultivated from the active lesions of leprosy may be divided into 5 groups

1 Partially acid fast or acid resistant diphtheroid organisms—the Babes Kedrowsky type. At least eighteen investigators have isolated microorganisms which apparently may be included in this group.

2 Acid fast organisms which produce yellow or orange-colored colonies. Five investigators have probably isolated organisms of this type. Clegg being the first to obtain a definite growth in pure culture.

3 Anaerobic acid fast organisms isolated by Ducrey, Campana and Serra.

4 Acid fast bacilli which do not produce colored colonies. Five investigators of whom Karlinski was the first have claimed to have obtained organisms of this type. Duval's recent work has been the most convincing regarding the etiological position of this organism.

5 Acid fast streptothrices isolated by Deycke, Pascha and Reschad, Bey and by Liston.

From a study of the literature one concludes that probably at least two—the diphtheroid and pigmented acid fast and perhaps all four varieties of these bacilli have been more or less commonly encountered in leprosy tissue. The diphtheroid organisms have been found in various parts of the world. In connection with the pigmented acid fast bacilli the experiments of Clegg and Duval are of interest.

showed fully developed leprosy 3 years later. Unfortunately for the value of the experiment the man was a native of Hawaii and had lepers in his own family. Against this experiment are the numerous instances where physicians have inoculated themselves and others with leprosy material with invariably negative results in Europe and Hawaii. Danielson inoculated himself and members of his staff with leprosy material and later Profeta performed other inoculations on 10 individuals but without success in a single instance. Vedder in the Philippine Islands also failed to successfully inoculate Philippino convicts. However DeLangen (1936) reports a case which he and Lichtenstein observed. A European living in the best surroundings in the middle of a European residential district was attacked by gall stones. The doctor who was summoned at once had just returned from visiting a leper to whom he had given an injection. He confused his syringes (a fact which he was later able to determine quite definitely) and gave the patient a morphine injection with the same syringe that he had just used for the leper without previously sterilizing it. Six months later there appeared on the forearm of the European just at the place where he had formerly had the injection a nodule from which a further skin affection spread to the surrounding peripheral tissues. Six months later lepra bacilli were found in a small piece of the nodule which had been removed for diagnostic purposes. In the second year a few small lepromata also appeared higher up the arm on his face back and legs in all of which lepra bacilli were found. After that energetic treatment was instituted. There was no further spread of the condition the lepromata that were present became slowly smaller in sections the bacteria diminished and finally entirely disappeared. In addition to this there then followed changes in the leprosy tissues which became typical connective tissue. The skin sensitivity was restored more slowly. In this case the incubation period was only 6 months which is much shorter than is usually accepted.

Lazoudaky on June 6 1936 allowed himself to be inoculated intramuscularly with 3 gm. of blood from a leper and 5 days later with a second inoculation of blood from another leper. On July 30 two small subcutaneous lepromas appeared. In August and September other lepromas developed and anaesthetic areas appeared within 5 months after the reinoculations. In 1938 he presented himself at the International Leprosy Congress as an example of positive infection after experimental self inoculation.

Accidental inoculation of physicians or attendants upon lepers with leprosy material on surgical instruments through cuts and abrasions of the skin have generally resulted negatively. However Rogers has reported 2 cases of doctors who wounded their fingers while operating on leprosy patients and both not long after developed leprosy commencing with anaesthesia in one and red patches in the other on the very fingers they wounded. In many cases however it seems evident that the simple implanting of the leprosy bacillus is not sufficient to produce infection. Probably intimate personal contact at certain infective periods of the

multiplication of the germs indicated that the ideal media and environment for the saprophytic existence of *M. leprae* had not been provided.

**Inoculation of Animals**—The many attempts made to transmit leprosy to animals have been unsatisfactory. Adler (1938) reported that he had been successful in infecting the Syrian hamster, *Cricetus auratus* with material from human leprosy nodules having previously removed their spleens. Evidence of multiplication of bacilli was obtained in 3 of the 4 animals used and a generalization in 1 of the 3. Burnet (1938) also records the successful infection of 1 of a series of hamsters with human leprosy. The spleen had not been removed. Five other experiments were negative. The infected animal was inoculated by the insertion under the skin of a small dose of a human leproma. In less than a year a subcutaneous lesion containing numerous acid fast bacilli developed. In this instance it would appear that at least an engrafting of leprosy tissue had occurred. Unfortunately this work has not been confirmed. Lowe (1939) in Calcutta, has inoculated with human leprosy material a number of hamsters some splenectomized. Half the animals were inoculated intraperitoneally with an emulsion rich in leprosy bacilli and in the other half a small piece of leprosy nodule was put under the skin of each. Two of the animals died within 8 months after inoculation, 12 were sacrificed 9½ months after and 3 a year after inoculation. In none of the animals was any macroscopic or microscopic evidence of a chronic infection found. Inoculations were also made in monkeys. Six were splenectomized and after the splenectomy wound had healed were injected intraperitoneally with emulsions rich in leprosy bacilli. Four of the monkeys died within 5 months of inoculation. In one of the animals, nothing abnormal was seen at postmortem and microscopically only a few acid fast bacilli were found in the omentum and mediastinal glands. In 3 monkeys the appearance was suggestive of a massive tuberculosis infection. It is well recognized that monkeys in captivity frequently develop a type of tuberculosis infection.

Sellards and Pinkerton (1938) report that the intracerebral injection of rat leprosy emulsions into monkeys and rabbits white rats and mice produces progressively generalized lesions. In monkeys only low grade infections were produced by human leprosy material. Rats infected intracerebrally revealed acid fast bacilli up to 3¼ years without progressive disease or active lesions developing. The infection of the mesenchymal cells of the reticulo endothelial system by rat leprosy in the organs and sheath of the nerves was demonstrated.

#### EPIDEMIOLOGY

Most authorities agree that every case of leprosy owes its origin to contact direct or indirect with some other case but exact evidence as to the manner in which the disease is transmitted or even the proof of transmission is to a great extent lacking.

In connection with its infectious nature frequent reference has been made to the inoculation experiment by Arning of a freshly excised leprosy nodule sewn into a skin incision of the arm of a condemned criminal. In this case a neuritis developed shortly after the inoculation and the patient

difficulty has been that there is no animal in which progressive leprosy can be produced experimentally and human experiment has generally failed except in rare instances

The two portals of entry that have been especially considered and seem to be the most likely are the *skin* and the *nasal mucous membrane*. In earlier years it was believed that the initial lesion of leprosy frequently occurred in the nasal mucous membrane. Pinkerton (1938) found that careful study of the mucous membrane will reveal that practically every leprosy patient has some nasal lesion due to leprosy. Del Rio (1936) showed that the nose was attacked in 82 per cent. Such lesions also frequently occur early in the course of the disease. However de Azevedo examined smears from the nasal mucosa in 59 persons who were in close contact with lepers but did not find acid fast bacilli in a single instance.

In this connection it is of interest that Waysor and Nassaga (1935) reported success in infecting rats with rat leprosy by intranasal instillation of rat leproma suspensions without traumatizing the nasal membrane.

The leprosy changes which occur in the buccal mucosa of human beings are found usually in cases of long standing and the evidence does not point to the mouth as a portal of entry. However when the mouth and pharynx are diseased large quantities of leprosy bacilli may sometimes be expelled from the mouth when the patient coughs or sneezes and it seems possible that the leprosy bacilli infection might occasionally be introduced into another individual in this manner. Pinkerton (1938) found that more than 20 per cent of leprosy patients had demonstrable infiltrated and nodular lesions of the tonsils. It also seems possible that such introduction might exceptionally occur from the inhalation of infected dust.

The *gastro intestinal tract* is apparently not favorable for the development of leprosy lesions. Black in the examination of the intestines at autopsy of 75 lepers many of the cases being well generalized infections failed to find leprosy lesions in the intestine. However whether the bacilli can enter the body through the gastro intestinal tract without producing visible lesions cannot be answered.

*Skin*—On the other hand Rogers and a number of other observers believe that the common mode of infection of leprosy is in all probability through accidental abrasion or through other lesions of the skin. Leprosy bacilli are being continually discharged from ulcerated nodules as well as from nasal lesions in at least 80 per cent of the nodular cases. These cases therefore are particularly dangerous as foci of infection. In the anaesthetic form the bacilli are obviously not given off from the nerve trunks and are only discharged in the nasal mucus in about 6 to 15 per cent of the cases.

The numerous attempts to inoculate man experimentally which have generally resulted negatively have already been referred to. However in this connection it must be emphasized that many individuals appear to be practically immune to leprosy and that man varies greatly in his susceptibility to the disease.

*Insects*—It has also been claimed that leprosy may be transmitted by flies, bedbugs, fleas, ticks, lice, itch mites or chiggers. Particularly during the febrile periods of leprosy *M. leprae* may circulate in considerable numbers in the blood and any blood sucking insect might ingest this organism.



disease, as well as special susceptibility influenced by poor nourishment and ill health on the part of the recipient may be necessary for the successful communication and acquisition of the affection

**Immunity**—There is little doubt that the susceptibility to the disease varies greatly in different individuals and it would appear that many healthy individuals are at least relatively immune to leprosy, and to successful inoculation. Thus leprosy runs a benign course in many who are apparently highly resistant and in these cases the lesions are usually present in the peripheral nerves. In highly susceptible individuals the disease often assumes a malignant course with rapid proliferation of the bacilli in the skin and internal organs forming the characteristic lepromata. Also in a considerable proportion of lepers (about one third of those in the Philippines) the disease becomes arrested that is clinically inactive without treatment. Generally the most infectious patients are those with numerous nodules and ulcerations.

Hopkins (1938) suggests that leprosy is not easily acquired by the average adult individual because of characteristics that are inherent in himself. There is some evidence that predisposition to the disease may be a hereditary family characteristic. As leprosy resembles tuberculosis more closely than it does other diseases what has been said of hereditary immunity in tuberculosis may be equally applicable to leprosy. Hereditary differences in individual resistance are in all probability of great importance in slowly elevating the average level of resistance of a race through the principle of survival of the fittest. Probably the rarity of leprosy among Europeans at the present time has been at least influenced by the operation of this principle. It is noteworthy also that in contrast to the disappearance of leprosy in Europe it has spread rapidly in the Hawaiian Islands where it did not exist until comparatively recent times. Its comparatively recent introduction in New Caledonia and the Island of Nauru was also followed by very rapid spread. In these instances we may have an example of races without hereditary immunity.

In practically all countries in which surveys of leprosy have been made the number of males has usually been more than twice that of females. This sex difference and incidence occurs in such widely separated countries and among peoples of such different habits and customs that the conclusion seems justified that females are inherently less susceptible than males and that they do not owe their immunity to accidents of environment or to less exposure to contagion or to their habits but rather to inherent feminine characteristics.

Hopkins believes that the Negro in Louisiana has a higher degree of resistance to leprosy than has the Louisianan of Caucasian ancestry.

**Method of Transmission**—Nothing definite is known of the method of transmission. This question is complicated on account of the long incubation period of the disease which McCoy believes is ordinarily at least as long as from 5-10 years and may be as long as 20 years. Hence when an epidemiological study is undertaken we are not so much concerned with the circumstances relating to the patient within a comparatively recent time but generally with those which existed some 4-15 years before. This is apparently one of the main reasons why we have not been more successful in tracing the source of infection in leprosy. Another

There are 2 types (1) of skin and muscles and (2) of the lymphatic glands

In the skin form areas of alopecia are present with thickening of the site invaded. These areas are most often on the back of the head. Just as in human leprosy the epithelium is unaffected, the corium however being filled with cells packed with acid fast bacilli, exactly similar to the picture in human leprosy. Ulceration of these subcutaneous nodules is common. In the glandular type the glands are enlarged and the lymph sinuses packed with the causative bacilli.

The disease is due to *M. leprae murum*. Morphologically it is indistinguishable from *M. leprae* and the lesions produced bear a close resemblance to those of the nodular type of human leprosy. Histological sections of the skin nodules show the same granulomatous infiltration in the corium and large rat lepra cells (histiocytes according to Olver) packed with bacilli.

The disease can be transmitted to rats of the same species and infection takes place as readily through skin abrasions as by subcutaneous inoculation. It is believed that the natural infection is acquired through the skin possibly from bites. Mechanical transmission by rat fleas is possible but no cycle of development has been demonstrated in any insect. The disease is not hereditary.

Attempts at cultivation of the organism have shown results similar to those obtained with *M. leprae*. Zinsser and Carey observed intracellular multiplication of the *M. leprae murum* in tissue cultures of growing rat spleen. Aside from this observation however the results reported are open to the same objections as those obtained in human leprosy.

Souza Araujo (1942) has studied anew the relationship between the organism of rat leprosy (*M. stefanski* and *M. leprae*) and has concluded that while the organisms are similar morphologically and that the pathogeny of both diseases is identical further accurate studies are necessary to explain their dissimilarities.

In spite of the similarity of human to rat leprosy most authorities believe that the diseases are distinct and that the organisms belong to separate species. There does not seem to be any connection between the disease in rat and in man as is the case with human and rat plague. The prevalence of rat leprosy in various parts of the world varies greatly. In Odessa 4 to 5 per cent of the rats were found infected and in Paris about 5 per cent while in San Francisco only one fifth of 1 per cent.

All efforts to transmit human leprosy artificially to various species of rats although they have been numerous have uniformly failed. Soule (1935) believing that a closer relationship might exist under natural conditions made an exhaustive study of wild rats captured in the Culion leper colony.

On this island one might think there was perhaps an ideal environment for the natural transmission of the human disease to rats since for over 25 years there has been ample opportunity for the rodents to come into intimate contact with contaminated material on account of the impossibility of immediately disposing of large amounts of infected human tissue from surgical dressing clinics and the operating rooms and elsewhere. Nevertheless not a single instance of rat infection was detected.

Muir Henderson and Landeman suggest that the relationship of human to rat leprosy is analogous to that of the human and avian tubercle bacillus.

**Infection through Contact.**—Long and close association with a leper is usually the history of the affected person. In countries where leprosy prevails it is not uncommon to find several lepers in one family and sometimes cases develop one after the other.

Denny in the statistical analysis of 10400 cases in the Philippine Islands found that 29 per cent gave a definite history of previous contact with at least one leper relative and McCoy in Hawaii and Gregory in Cape Colony found 37 per cent gave such a history although the compulsory segregation laws made the patients loath to

Thus Rudolph found the leprosy bacillus in the intestines of a tick which had sucked blood from a patient suffering from nodular leprosy for as long a period as 13 days. Lutz (1936) still believes that the mosquito is the transmitting agent in leprosy. Valverde however has pointed out that there is a marked lack of experimental support in the evidence presented by Lutz. The case reported by Jeanselme in which the individual had been born and lived all his life in Paris probably excludes the mosquito as being the only means of infection. Lebouf found leprosy bacilli in the stomachs of flies which had been feeding on leprotic ulcerations and did not find acid fast rods in flies which had fed on persons with nerve leprosy or upon those not showing open lesions. He thinks that flies may deposit faeces containing bacilli about the nasal orifices or upon wounds of well persons bringing about thereby their infection. Lamborn has shown that the Muscid fly *Musca sorbens* may take up from an infected medium and later deposit *M. leprae* up to 24 hours. After regurgitation has taken place from an infected meal and the vomit drop has been withdrawn the leprosy bacilli may be laid down on whatever food material (as an exudate over abrasions of the skin) to which the insect may happen to apply its proboscis shortly afterwards. Marchoux has shown that in the case of rat leprosy flies can only transmit the disease mechanically. In fact in relation to the transmission of leprosy by insects it may be said that the evidence is not convincing though in some instances it seems possible that transmission of the bacilli might rarely be accomplished by some of these insects.

There is a firm conviction in the minds of many observers that leprosy is spread by sexual intercourse. In Nigeria, Madagascar, and China many of the natives firmly believe in leprosy being contracted in this manner and leprosy bacilli have been found in the semen and in lesions of the penis and vulva in lepers. Obviously however this is not the only method of spread since the disease is often observed in young children.

In the Hawaiian leper colony it was found that of 98 healthy residents who lived with diseased wives only 5 developed the disease and of 83 healthy wives who lived with diseased husbands only 4 developed the disease. It is generally acknowledged that congenital leprosy is very rare but on the other hand the children of leprous parents frequently develop the disease when they remain with their parents.

**Vaccination**—It was formerly claimed in Hawaii that vaccination against smallpox has been a means of the spread of leprosy. While this might be a possibility if human lymph infected with leprosy bacilli was employed obviously when bovine lymph is used there could not even be a chance of occasional infection.

Thus although the exact method of transmission of the disease is not known a number of these possible means of transmission must be borne in mind. Obviously leprosy may be transmitted in more than one way and possibly in several ways.

**Diet**—Hutchinson's theory that the disease bore relation to the eating of fish, or of salted or spoiled fish has received no important support in recent years nor has there been recently any important evidence submitted which points to infection with leprosy through the alimentary tract.

Dietary deficiency may be a determining factor in susceptibility to leprosy, but we have little definite information upon this subject. However it is well recognized that the disease is often closely associated with poor diet and that an amelioration of the symptoms of the disease frequently occurs when the diet is generous and well balanced.

**Rat Leprosy**—A disease occurring naturally in Europe, Asia and America among rats was first observed by Stefansky in Odessa in 1903.

The extent to which leprosy occurs in *family* groups suggests a hereditary familial lack of resistance. In some localities the rate of family infection is higher than others. McCoy in Hawaii, Gregory in Cape Colony and the Leprosy Commission in India found that the proportion of healthy persons living with lepers who became infected is 42.45 and 55 per cent respectively in these different countries while in Japan and Norway the percentage in both was about 27. Even between infected husbands or wives not usually over 5 per cent of adults contract the disease the single exception being in India where the percentage reported is 65. The comparatively small number of cases occurring in husband and wife may again be an example of a high degree of resistance in the uninfected spouse.

Rogers studying 700 cases came to the conclusion that about one fifth of these lepers got their infection from conjugal or other similar close contact. Two fifths had a history of living in the same house. One fifth took care of lepers and in the remaining fifth there was a history of close contact as child playmates. There were also occasional records of wearing a leper's clothes or having a leper woman as wet nurse.

Thus leprosy cannot be regarded as a highly contagious disease. It has been maintained that an important factor in the belief that it is at least feebly contagious is based on the disappearance of the disease in Europe following isolation of the lepers. A striking instance is that of Europe in the thirteenth and fourteenth centuries where with 20,000 leper asylums for isolation the disease practically disappeared by the fifteenth century. In Norway there were 2850 cases in 1856 while in 1907 there were only 438 left. At the end of 1913 there were only 284 cases, 181 of these being interned and 104 in their own homes. Finally in 1937 there were only 18 cases. The reduction was attributed to isolation. However this reduction might have occurred independently of isolation because Hansen in investigating the descendants of 160 known Norwegian lepers who immigrated to the Northwestern States of America was unable to find a single leper among their descendants.

This and other facts militate also against the views that leprosy may be inherited and the idea is generally held that if a child be taken away from its leprous surroundings after birth there is little or no likelihood of its developing leprosy. The separation of the child from its mother should be as soon as possible. Rodriguez having shown that if this is delayed for 6 months it has usually acquired the infection.

Prenatal transmission of leprosy from parent to child can not be regarded as a cause for the prevalence of leprosy in families.

*Congenital leprosy*.—In spite of the fact that leprosy bacilli have sometimes been found in the placenta, foetus and milk of leprous women the children of leprous patients are generally born healthy. Zambaco states he has seen congenital cases of leprosy and Nakayo has reported a single case in Japan of a newborn infant with typical leprous infiltrations and leprosy bacilli. However these are very unusual exceptions and there is much evidence showing that prenatal transmission of leprosy from parent to child is of such rare occurrence that it may be regarded as a negligible cause of transmission. While children of leprous parents develop the disease much oftener than the children of healthy parents among the same population the children born in leprous families are not nearly so likely to develop the disease if removed at once from the leprous

acknowledge infected relatives. In South Russia Debio found that 60 per cent of the lepers acknowledged contact with other lepers while Kereval in the Caucasus found 89 per cent gave a history of contact.

Two cases have been reported which especially show that those who live in close relation to lepers may develop the disease. In one a leper returned to Ireland and his brother who had never been in a leprosy country but who had occupied the same bed with the leper and worn his clothes developed the disease in about five years. A similar case is reported from Germany.

On the other hand, in other instances where contact has apparently given the most favorable opportunity for infection between the diseased and the healthy as is often the case in leper colonies, the disease is rarely contracted.

As regards those living for a long time in attendance on lepers there have been only a very few instances of the contraction of leprosy as in the case of Father Damien at Molokai and two instances in Sisters of Mercy. Such cases however are most exceptional as the hundreds of attendants on the unfortunates continue their work for years without showing any signs of leprosy.

It is stated that there has never been an instance of transmission of leprosy to any attendant at the Saint Louis Hospital Paris. Anderson (1936) reported that 17 individuals employed as attendants at the Paloseco Leprosy Colony in Panama for as long as 20 years in some instances do not in any case show any evidence by clinical or laboratory tests of having acquired leprosy. Hopkins (1938) also points out that the personnel of the national leprosarium at Carville numbers 199 and that there has been no instance of leprosy developing among them nor are there any instances in the personnel in the former leper home of Louisiana. Included in the personnel were Sisters of Charity which in 1938 numbered 19. The Sisters have been in attendance upon the lepers as nurses at this institution during the last 41 years.

The belief now generally accepted that leprosy is only feebly contagious might be a satisfactory explanation for the fact that no cases have developed in the attendants were it not for the observation that in the familiar relation leprosy may appear sometimes to be more than feebly contagious. It is true that precautions are taken at Carville to safeguard the personnel and it may be true that these precautions alone have been sufficient for the protection of the attendants. While some of these attendants may be less susceptible to leprosy it does not seem that one would be justified in assuming that all of them during 41 years were immune. Hopkins suggests that such resistance to the disease may be due in part to inherited immunity.

As showing that in some localities even with intimate contact infection is rare it is stated that of 225 healthy Hawaiians living in the same houses with lepers only 4  $\frac{1}{2}$  contracted leprosy. Even when married to lepers only 9 out of 181 healthy people contracted leprosy from their leprous mates.

In Japan 7<sup>00</sup> of children of lepers contract the disease 3 8<sup>00</sup> of those married to lepers and 7<sup>00</sup> of people living in the same house with lepers. Just as with tuberculosis in which all evidence points to the predominance of infection in early life and its infrequency in adult life so is it true of leprosy. Among 10 000 lepers in the Culion leper colony Denny notes that 35<sup>00</sup> were brothers and sisters 27<sup>00</sup> were cousins 11<sup>00</sup> were children of lepers 7<sup>00</sup> parents of lepers and only 1<sup>00</sup> husband and wife. This would indicate that the relationships involving intimate contact in childhood are etiologically most important.

their associates or members of their families making a second generation on American soil. He thinks that it is plain that in spite of the heavy importation of lepers the Northwestern States have not furnished a favorable location for the transmission of the infection.

Another example illustrating the non infectivity in most parts of the United States is found in the experience of New York City. With a single doubtful exception McCoy was unable to find any record even suggesting that anyone has ever been infected with leprosy in New York City although over a period of a few decades it is known that hundreds of lepers have lived there for varying periods of time. He points out that this geographic distribution of infectivity is one of the most interesting features in connection with the epidemiology of leprosy.

Flandin has recently conducted a careful study of the origin and mode of contagion of 95 lepers in Paris. He found that only 6 had contracted their disease in France. He concluded that under ordinary social conditions in France the danger of contracting leprosy is too slight to necessitate segregation.

However in other localities as for example in the Philippines Rodriguez (1938) has shown that where the poor have to live in houses with one room without facilities for isolating and caring for a contagious patient the spread of the disease by contact is common in the home. Statistics show that in about one half of the cases in Cebu the disease is acquired within the home. In Cairo the proportion of home infections varies in different regions from 25 to 75 per cent of the total cases.

It is not clear to what extent climate may influence the disease since it extends to all climates and all latitudes.

Rogers has stressed the importance of excessive humidity as favoring the spread of leprosy in the tropics. The only tropical areas with little or no leprosy are the very dry ones with rainfall under 10 inches. Again humidity keeps the skin bathed in perspiration which favors mould and bacterial infections—the skin in these conditions perhaps sometimes offering less resistance to the entrance and growth of leprosy bacilli.

Maxwell (1937) does not attribute any important influence to the climatic causes in China. He points out that leprosy is very prevalent in the low lying delta of the West River in Canton Province which lies within the tropics and is hot and steamy in the summer. However the disease is quite as prevalent in the uplands of Yunnan where much of the country is at a level of 4000 feet above the sea and where the climate is salubrious. In the high mountains of the Tibetan border it is also very prevalent. He has seen many cases at altitudes of over 5000 feet. In one such region there is a valley in the high mountains among the perpetual snows which is known as the leper valley. However such studies do not reveal in what climate the disease is most likely to originate. Leprosy is also prevalent in eastern Tibet. Maxwell has found that it prevails in almost every variety of climate. In parts of the Province of Shantung which are the most heavily infected regions in China the climate is hot and dry for much of the year and the soil is very sandy.

#### PATHOLOGY

**General Pathology**—In discussing the pathological changes it may be recalled that leprosy is essentially a chronic disease in which the bacilli

surroundings and it seems evident that there is not the same tendency for the children to contract the disease from their parents or from other lepers if they are separated from them shortly after birth. The Hawaiian statistics show that some 15 per cent of the children of leper parentage develop leprosy when they remain with their parents.

**Race, Age and Sex**—Whether race predisposes in itself to the disease seems doubtful. However leprosy occurs more frequently in Orientals, Polynesians and Africans of the poorer classes. The conditions under which these people live undoubtedly expose them more frequently to infection since uncleanness, overcrowding and generally poor conditions of life and diet favor the transmission of leprosy.

The disease originates particularly in youth and early adult life, children being much more susceptible than adults. However cases are rare in very young children and the disease is also uncommon after 70 years of age, the majority of cases occurring between the tenth and thirty-fifth year.

McCoy tabulated the ages of 1,058 lepers at the time of admission to the *Molokai Settlement in Hawaii* and found its peak to be in the 16 to 20 year period. Doull, Rodriguez and their associates found in a rural area in the Philippines that in more than half of the patients the lesions were noted before the age of 15. Maxwell in China, found nearly two thirds of the cases occurred from 10 to 30 years.

**Sex**—The number of males attacked with leprosy in different parts of the world is generally more than double that of the females. Apparently there is no conclusive evidence of why this is so. Hopkins maintains this is due to the fact that females are actually less susceptible than males. Doull (1938) points out that in childhood there is apparently no difference in incidence as between the sexes and that among the children born at the Culion Leper Colony in the Philippine Islands the proportion of females developing leprosy is about equal to that of the male. Rogers and Muir suggested female lepers may die at an earlier age on the assumption that the female is less resistant to the progress of the disease. Studies made in the Philippines have not substantiated this view. Maxwell (1937) found in China 95 of his patients had leprous fathers and only 45 had leprous mothers.

**Climate**—McCoy (1938) points out that in the United States, excluding imported cases, leprosy appears to be infective to an appreciable extent only in localities in states bordering extensively on the Gulf of Mexico. Cases originating elsewhere are rare. Nearly all of the cases (excluding those that are imported) acquire the infection in Florida, Louisiana or Texas and apparently mainly in certain parts of these states. It is exceedingly rare for persons to acquire the disease in any other part of the country. He points to our experience in certain of the Northwestern States. About 200 Scandinavian lepers, either in the incubation stage or with developed symptoms, came to the United States settling chiefly in Minnesota and adjacent states in the latter half of the 19th century. These 200 cases gave rise to less than a dozen cases among

of dehaemoglobinized venous blood in 84.31 per cent of cutaneous and mixed cases of leprosy bacilli. On the other hand in nerve leprosy examination of the blood is generally of little value and the leprosy bacilli are rarely present.

Sections of the nodules of leprosy when stained with carbolfuchsin and examined under a low power of the microscope frequently show



FIGURE 1—Histological appearance of leprosy nodules. The epidermis shows a thinning out with obliteration of the interfollicular spaces. Many of the follicles are vacuolated and the dermis contains these cells and masses of leprosy bacilli. (Army Medical Museum No. 43690)

reddish patches due to the masses of closely placed leprosy bacilli. It is particularly on account of the relationship and number of the bacilli in the lesions that we regard the leprosy bacillus as the cause of the disease.

**Pathological Histology**—The lesions of the skin may be divided for purposes of description into 3 types: the macular, tuberculoid or reacting macules and the lepromatous. Sections of macules may show no changes in the epidermis unless hypopig-



develop rather slowly. Not until the organism is present in large numbers do the local effects produced by the bacilli become visible. Later when the local lesions may be extensive and marked changes in the tissues present general toxic phenomena are often scarcely apparent. Also there are no secondary lesions in the kidneys or other organs with the exception of the nerves in some cases that suggest the formation of a circulatory toxin and while there is no evidence of immunity being produced by the leprosy bacillus there also may be little impairment of the general health. It therefore seems clear that the toxins produced by the leprosy bacilli are very mild and are formed slowly and that it is particularly the actual presence of the bacillus that gives rise to the specific lesions of the disease. The ulcerations and necrosis that frequently occur are apparently secondary to the activity of the bacilli themselves.

The leprosy bacillus develops more commonly in the skin of the face and of the extremities and in the nasal mucous membranes, but it may affect almost any portions of the body with the exception of the muscles, bones cartilages and intestinal tract. It is very abundant in fluid expressed from the nodular leprous lesions and in the ulcerations of the skin and is often found in the sputum as well as in the nasal mucus. In the anaesthetic areas of the skin it is usually not found but in such cases the bacilli are frequently observed in the nerves which supply these areas lying sometimes both between the fibers and within the nerve cells. The bacillus is also found particularly in the enlarged lymphatic glands and in the testicles. In the internal organs it is particularly prevalent in the liver and spleen see Fig. 191.

There has been some difference of opinion expressed about the occurrence of leprosy bacilli in the circulating blood but from personal experience the writer feels as do others that the organisms may occasionally occur in the circulating blood particularly during the attacks of leprous fever. The bacilli are often present in fairly large numbers in the endothelial cells which line the blood vessels and they occasionally invade the fibroblasts which are next to these cells apparently through direct extension. There is no reason to think that the bacilli may not sometimes also extend through the endothelial cell wall into the lumen of the blood vessel.

It has been suggested that reference to leprosy as a bacteraemia is at least generally erroneous. In support of this view Soule (1934) at the Cullen leprosarium cultured over 500 specimens of blood with negative results and stained preparations of over 1000 specimens of blood all of which were negative for acid fast bacilli. Since *M. leprae* is almost invariably present in cases of the disease even in the apparently healthy skin their presence in a blood preparation might be accounted for by obtaining the blood through the skin. Obviously the failure to cultivate leprosy bacilli from the blood has no significance on account of the very great difficulty of obtaining a growth from any of the lesions. Also all of the cases in which the blood was examined by Soule were under treatment with iodine esters of *Hydnocarpus wightiana*.

However after dehaemoglobinization a great many reliable investigators have demonstrated leprosy bacilli in the blood. Radna (1938) reported positive bacteriological results in the examination of the sediment

among the connective tissue fibres which gradually develop. It is thought that an axonal degeneration involves the cells of the anterior horns so that this as well as the peripheral neuritis is a factor in the muscular atrophies which are features of the disease. The sensory fibres are destroyed before the motor ones.

**Lesions in Other Tissues**—Leprous changes are common in the anterior part of the eye as the conjunctiva, cornea and iris but rare in the posterior eyeball. The first changes to be noted may be inflammatory reactions of the sclera and cornea characterized only by redness. Later infiltration occurs and the cornea may gradually become opaque. Eventually the anterior aspect of the eyeball may appear as an undifferentiated mass of granulomatous tissue and total blindness result.

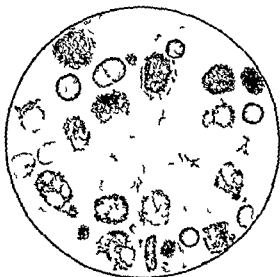


FIG 191.—Section of placenta showing leprosy bacilli (X 800) (By permission of the Ministry of Health, London)

The mucosa of tongue, larynx and pharynx is often involved. The cartilaginous septum of the nose is frequently perforated and partially destroyed through pressure of the granulomatous tissue. The testis and ovaries may show marked connective tissue increase and in advanced cases be converted into a fibrous mass, the fibrous tissue reaction being more intense than in the skin. Large numbers of leprosy bacilli are usually present. Next to the skin, the mucous membranes and nerves, the lymphatic glands are most frequently affected. The nodes become moderately enlarged but do not tend to break down. On cut surface there is usually a yellowish discoloration. Bacilli are often found in them without difficulty and there may be accumulations of lepra cells.

The liver and spleen are often enlarged. Lesions are often present in the liver and spleen which are barely visible to the naked eye. The lesions which may be recognized consist of minute pin-point areas, appearing somewhat like dust particles sprinkled on the cut surface. Amyloid changes frequently occur in the late stages of the disease. Histologically the main lesions consist of cellular infiltrations with local accumulations of lepra cells. Lymphocytes are present in small numbers, with fibroblastic proliferation.

mentation is present. Beneath the epidermis of the corium areas of lymphocytes and monocytes with slight fibroblastic proliferation may be present. The accumulations of monocytes are characteristically perivascular. The cellular reaction is usually present about the hair follicles and sweat glands and intense enough to have interfered with their function. Lepa cells are not present in these lesions and bacilli are difficult to demonstrate. These lesions may later undergo lepromatous changes at which time the lepra cells make their appearance.

In tuberculoid leprosy a more intense cellular reaction occurs the epidermis is thinned and the interpapillary pegs often obliterated. Focal accumulations of leucocytes beginning close to the epidermis occur. Cellular reactions are present around the capillaries in the central areas epithelioid cells may be present with a diffuse lymphocytic infiltration and the appearance of fibroblastic proliferation. Giant cells resembling those of the Langhans type may be present but are by no means constant. Leprosy bacilli are frequently difficult to demonstrate and often are not found until the lesion assumes the nodular type.

The most characteristic lesion in leprosy is the granulomatous nodule or leproma which is formed in response to the presence of the leprosy bacilli. Incision of such a nodule often shows a smooth glistening surface of a yellowish to slate gray color. In whatever way introduced the leprosy bacilli tend to invade and multiply in the lymphatics of the corium and subcutaneous tissue. In response to irritation large monocytic cells appear and phagocytize the bacilli in large numbers so that eventually the outline of the cell as brought out in acid fast staining is that of a mass of red bacilli. These red staining bodies are called lepra cells. With the accumulation of these lepra cells the epidermis is pushed outward into a nodular protrusion the epidermis becoming markedly thin and the papillae obliterated.

Endothelial cells also phagocytose the bacilli and these bacilli together with the free lying masses of bacilli in the lymphatic sinuses constitute so called globi when seen in transverse section. The toxicity of the lepra bacillus is evidently only slight. Very large giant cells of the Langhans type may be produced. This slight toxicity probably explains the absence of caseation in leprosy. The capillary walls are invaded and often their lumina occluded. The arteries of the leproma as the granulomatous mass is termed undergo an arteritis with thickening of their walls.

The leproma of the skin consist of a mass of cells of varying sizes and types in a connective tissue framework. Infiltrations are chiefly about the hair follicles, sweat and sebaceous glands and arteries. The epidermis is separated from the leproma by a connective tissue layer and is uninvolued except for a thinning out of the layer and obliteration of the interpapillary epithelial pegs.

In nerve leprosy the affected nerves are swollen and reddish gray in color. Cellular proliferations in the region of the blood vessels and later in the perineurium and finally the endoneurium cause pressure on the axis cylinder with consequent degeneration. Lepa cells are not commonly seen in these lesions and bacilli are not usually demonstrated. However they are found frequently enough to associate the nerve lesions with the bacterial invasion. In the long standing lesions fat cells appear

In some instances the incubation period may extend over many years. Hallopeau recorded a case where the disease did not develop sufficiently to be recognized for 21 years after the patient left the infected district. Other cases of long and indefinite periods of incubation have been reported.

*The early manifestations* are vague and indefinite, consisting chiefly of malaise, weariness and mental depression.

As might be expected in a disease of so long an incubation period the onset of definite symptoms varies considerably. Usually the onset is slow and insidious. The patient may first notice a patch of skin in which some slight change in color or some thickening appears, or the attention may be first drawn to lack of sensation. Anaesthesia is a most important symptom. If the hands are first affected the loss of heat or cold sensation may be first noticed, the patient unwittingly burning or blistering the skin which has touched some hot vessel or other substance. In other instances the attack may begin with neuralgic pains followed later by anaesthesia of the skin. The occurrence of these anaesthetic patches of skin sometimes with thickening of the nerve in the vicinity early in the disease is often of great value in connection with the diagnosis. In some cases contracture or atrophy of some of the muscles of the hand or feet may be the first noticeable symptom. In other cases the definite onset of symptoms may be more acute with more or less severe fever and the appearance of nodules in the skin. These nodules may be single or may vary in number to a dozen or more. Sometimes with the fever and before the appearance of definite nodules an erythematous condition of the skin, usually bilateral and symmetrical, occurs. The color of this eruption may be pink to port wine in color, occasionally it is fawn colored and then more characteristic. The patches at first are evanescent. Later they return with the fever and there may be successive attacks of these symptoms. Gradually, however, the skin becomes thickened in the erythematous patches indicating a deposit of leprosy bacilli. In other cases the nervous symptoms and areas of anaesthesia may show themselves in some of the sites of the former erythematous areas. Still in other cases the onset may be more subacute in character with fever and severe pains especially in the limbs, the condition developing into a neuritis in which the patient is sometimes unable to move the limbs especially on account of the pain. In some cases the formation of crusts in the nose and nasal obstruction may be the first noticeable symptoms.

Special symptoms that are often noted are (a) irregular accessions of fever (leprotic fever) attended with rather profuse sweating so that the onset may be mistaken for a malarial infection, (b) progressive weakness, the patient being easily fatigued with a tendency to somnolence, (c) alternating attacks of dryness and hypersecretion of the nasal mucous membrane with frequent attacks of epistaxis and (d) various neuralgic manifestations or paresthesias as well as headache. These prodromal manifestations usually precede but may accompany the outbreak of the spots.

Leprosy bacilli are ordinarily present in large numbers. Miliary lesions have also been observed in the lungs and in some instances the writer has found it impossible to distinguish such lesions from miliary tubercles. Black (1938) in a study of 75 necropsies, believes that whenever the gross pathology of the lung had been associated with acid fast bacilli and the organisms found were subjected to cultural and animal experimentation with one exception they had proved to be tubercle bacilli.

Nephritis is common in leprosy and the bacilli are sometimes present in the kidney parenchyma and occasionally lepra cells are present. However leproma are not produced.

It is presumed that the chief mode of spread of the bacilli from one organ to another is through the blood and lymph. Since the lymph nodes harbor the bacilli often in large numbers there seems to be little doubt that spread through the lymphatics may take place. On the other hand, bacilli are often detected in the blood stream and this taken together with the early perivascular lesions also points to the blood stream as a channel for the spread of the infection.

#### SYMPTOMATOLOGY

**Incubation Period**—The incubation period of leprosy has been variously stated to vary from about 1 year or less to 10 years or more. In a few instances the incubation period has been given as a few weeks. McCoy suggests that the average incubation period may be about 2 years. In cases which have shown the longer periods of incubation the disease may have existed in latent form for some time or slight symptoms may have been overlooked as might very well occur in the anaesthetic form of leprosy.

Rogers has especially studied the length of the incubation period and concludes that it depends very largely on the mode and length of exposure to infection or on the dose of the virus and the frequency of its repetition. He states that under certain conditions of direct inoculation the incubation period may be as short as 6 months. With close contact such as sleeping in the same bed as a leper the average period is 20 months. With less intimate contact such as would arise among those dwelling in the same house it has proved to be a little under 3 years. Lastly where the contact is less close still mere association as for example where children are allowed to play with infected children 5 years may be regarded as an average.

The great susceptibility of children to leprosy is well recognized. Goodhue and Hasseltine (1934) have reported the development of leprosy in a child of leprous parents at the age of 19 months. This child had been removed from leper surroundings within 6 hours of birth and had not since been in contact with lepers hence the incubation period of 19 months seems definite. Henry (1924) has also reported a case in a child aged 3 years who had been separated from his leper parents months after birth. The first sign was a red patch on one thigh which later enlarged and showed anaesthesia. The exposure of infection was therefore only 2 months and the incubation period 3 years.

nervous type. However this proportion varies considerably in different countries. At one time a classification of the 239 lepers at San Lazaro Hospital Manila P. I. showed 97 cases of nodular, 42 of nerve and 93 of mixed leprosy with 2 cases of doubtful nature. The nodular disease is usually more readily recognized than the anesthetic neural type.

### NODULAR LEPROSY

**A Typical Case**—After more or less indefinite and uncharacteristic prodromata the definite onset is by an outbreak of brownish red spots or



FIG. 192.—Nodular leprosy.

macules which later become pigmented and thickened. Sometimes only one macule may be present at first. The spots are at first erythematous and tend to come out in crops attended with attacks of irregular fever. They soon have the appearance of limited areas of sunburn. They vary in size from 1 or 2 millimeters to a patch the size of the palm of the hand. The macules develop in size by peripheral extension. They are raised and have a preference for appearing on the lobes of the ears, the nasal alae, the forehead, eyebrows, cheeks and chin. The extensor surfaces of the forearms, thighs and buttocks are also favorite sites for the indurated spots. The palms of the hands, soles of the feet, hairy scalp, groin and axillary regions are almost never attacked. These spots may be hyperaesthetic at first but soon show loss of pain and

It is the prominence of the nasal manifestations that caused Sticker to insist that the primary lesion of leprosy is of the nasal mucosa. Kedrowsky (1935) is inclined to accept this view but believes the lepra bacilli can penetrate through the mucous tissues without causing any demonstrable pathological changes or primary lesion. The general view however is that Sticker's belief is without sufficient foundation to definitely establish it.

Some have recently suggested that the disease first manifests itself in the lymphatic glands punctures of such structures showing bacilli rather frequently although in less proportion than upon examination of the nasal mucosa. In fact surveys made in Hawaii and in Brazil showed the nasal mucosa invaded in at least 82 per cent.

In Culion careful studies have been made of 300 children living with their leper parents as to the earliest signs of the appearance of leprosy and in not a single case has a primary lesion of the nasal mucosa been noted. Of 24 cases contracting the disease which first appeared in skin areas only 13 showed nasal lesions. Muir in studying the first appearance of lesions of the skin in 1056 lepers in India found the early lesion to be present on those skin surfaces which would naturally come in contact with the bed while sleeping. These were cheeks outer surfaces of extremities and buttocks. The flexor surfaces the neck and the middle of the body were nearly exempt from primary lesions. Rodriguez at Culion in 45 cases has found in about 5 out of 6 the first signs of leprosy to be single spots but in the remaining one sixth the disease may start as a generalized rash. The primary lesion was on the buttocks in 28.8 per cent on the cheeks in 11.8 per cent on the legs in 15.3 per cent on the arms in 10.2 per cent but never on the chest and abdomen. Anderson and his associates (1936) in Brazil found the earliest lesions observed upon the lower extremities in one half of the cases.

As to characteristics of early lesions Forman studying 252 cases gives the following percentages pigmented anaesthetic patches 74.6 erythematous red patches 11.9 erythematous anaesthetic patches 2 depigmented non anaesthetic patches 9.5 paralysis in 5 and ulcers in 1.5.

Labernadie has pointed out that in the early macular stages percussion of the long bones may be painful and that loss of the outer third of the eyebrows occurs. Irritability and enlargement of the ulnar nerve is common and he found that neural involvement was next most frequent in the subcutaneous branches of the cervical nerves.

**Phases and Stages of Leprosy**—Muir recognizes 3 phases in the development of leprosy (1) a phase of quiescence in which the bacilli multiply but without general symptoms (2) the phase associated with inflammatory local lesions and general toxic symptoms and (3) a phase of subsidence of the reactions of phase 2.

He also recognizes 3 stages in the progress of a case (1) with few bacilli limited lesions and no palpable skin thickening (2) with enormous multiplication of bacilli and rapid spread of skin lesions all over the body and (3) subsidence of the lesions of the second stage, with a tendency to granular degeneration of the bacilli. There is often regression. The lesions in individuals who appear resistant not infrequently undergo rapid resolution and may disappear. Leprosy is most amenable to treatment in the first stage next in the third stage while in the second stage treatment may aggravate the disease.

It is usually reported that in northern climates nodular leprosy forms about 70 per cent of cases while in the tropics the larger proportion is made up of nerve leprosy. Muir with a wide experience in India has found that approximately two thirds of the cases of leprosy in cooler climates are of the nodular type and two thirds in hot climates are of the

The eye is involved with great frequency in this form of leprosy infiltrations of the eyelids conjunctivæ cornea and iris occurring with subsequent ulcerations and loss of sight.

The nodule on the face backs of the hands buttocks etc may disappear by resolution but the tendency is for them to ulcerate and produce various contractures and deformities. When an ulcer is formed it seldom heals before the whole of the lepromatous tissue has been shed. The patient as a result of this often becomes much disfigured. The mucous membranes of the nose and mouth are also likely to become the seat of infiltration which later necroses and leaves chronic ulceration. Frequently extensive scars form particularly where the ulcers have been about the mouth and the mouth as a result may become so contracted that feeding may be difficult. Many of these cases die from simple exhaustion. In a number great pain is suffered in eating and swallowing owing to the ulcers of the lips tongue and throat. As a result of disease of the larynx and throat the voice often becomes croaky and sometimes reduced to a whisper a very characteristic feature. The patients also often suffer with painful dyspnoea. The bones are sometimes involved. There may be a specific *periostitis* with or without exostosis formation. Necrosis may also occur.

The glands in the region of the lesions become enlarged but do not tend to suppurate. Visceral involvements are not common but miliary lesions of the liver and spleen have been reported. A much disputed question is that of leprotic involvement of the lungs. It is probably rare but does occur and in the instances of finding acid fast organisms in the sputum of a leper one should perform inoculations of guinea pigs to determine if the tubercle bacillus is present.

The course of the disease is essentially chronic and if some intercurrent affection does not carry off the patient the end comes usually from a cachectic condition after a number of years the temperature gradually falling and a state of somnolence ushering in the end.

When nerve leprosy develops in a case of the nodular type the progress of the nodular lesions is sometimes interrupted and the course of the disease apparently prolonged.

### NERVE LEPROSY

**A Typical Case**—The prodromal manifestations are characterized by the results of irritation of the granulomatous tissue upon the nerve fibers and are chiefly neuralgic pains or signs of sensory disturbances as formication paraesthesia etc. In particular are the ulnar anterior tibial peroneal and facial nerves attacked the process very rarely extending above the knee or elbow. More rarely the median radial brachial great auricular and cervical nerves are involved. Especially when the nerve passes over a bone and lies close under the skin can the thickening be felt.

Anaesthesia of the region supplied by the ulnar nerve with contractures of the fourth and fifth fingers may be signs directing our attention to the



temperature sense with retention of touch sensation (dissociation of sensation) The spots do not sweat they remain dry even in a general perspiration

The areas of infiltration may increase in size or undergo superficial erosion which may after healing leave pigmented scars In other instances they may gradually disappear Later in the disease sometimes following successive febrile accessions a reappearance of either macules or so called leprous nodules appear which mark another stage of the disease These are reddish brown and when first seen are often about the size of a pea, but they gradually enlarge often to the size of a pigeon's

egg, and sometimes larger They usually appear as tense shiny masses reddish brown in color, and may remain single or several coalesce With each fresh group fever usually appears Sometimes the nodules are superficial and sometimes they seem to protrude from the skin surface At other times they lie deeper and can be felt under the skin but not seen Infiltrations then occur in the deeper layers of the skin and subcutaneous tissue On palpation of the skin, it feels definitely thickened In other cases the nodules may lie so deep that the skin can be rather freely moved over them and in these situations they sometimes become enlarged to the size of a pigeon's egg When the more superficial nodules are grasped between the fingers they are often found elastic



FIG 193 —Leonine facies in nodular leprosy (After Nocht)

to the touch As the result of active sebaceous secretion they may have a greasy appearance

As the disease progresses the nose becomes thickened and particularly the skin about the face and cheeks The thickened indurated skin frequently assumes a swollen glossy appearance the natural lines and folds being exaggerated giving the face a leonine appearance hence the name leontiasis or that of a satyr hence satyriasis With the development of the nodules a striking feature is that the hair falls out of the affected areas As the face particularly the superciliary region is prone to leprous eruptions depilation of the eyebrows is often an early and very characteristic phenomenon The beard too is apt to be patchy On the other hand, the scalp is never or very rarely, affected with leprous eruptions or alopecia

Nodules may develop in the mucous membranes of the nose mouth and larynx giving rise to foetid discharges or epistaxis and obstruction of the nares difficulty in mastication as well as in breathing and a raucous voice

manifestations of nerve leprosy. The nails are not generally lost but they may become rough or atrophied into hook like appendages.

It is at this stage of the disease that the nerve trunks begin to enlarge especially the ulnar at the elbow and the great auricular as it crosses the



FIG. 9.—Nerve leprosy showing deformities of the fingers and hands. (From U.S. Navy Medical Bulletin.)

sternomastoid muscle. The characteristic nerve enlargement is spindle shaped or beaded. Sometimes they are hard and cord like on palpation. These nerve enlargements are at first tender but later become painless and there develop areas of anaesthesia and trophic changes of the skin and

true nature of the disease and in those cases where the appearance of smooth yellowish brown spots precedes the neuritic manifestations we may here also find anaesthesia provided the eruption has lasted for some time

In brief the fully developed case of nerve leprosy shows anaesthetic spots trophic lesions of the skin and bone together with muscular palsies. The spots often appear singly and may vary from 1 to 10 cm. or more



FIG. 194.—Maculo-anesthetic leprosy

in diameter. They are not raised, have a sunburnt color, and do not sweat. Instead of having a preference for the exposed parts they most frequently appear on the covered portions of the body or limbs as trunk, buttocks scapular region thighs or arms although the first appearance of spots may be on the face.

These spots often resemble the lesions of ringworm as they have an erythematous border with a pale center but they are often oval in outline rather than round, and there is no scaling. In due time the skin of anaesthetic patches tends to atrophy losing the hairs and glands. Eventually it may become so thin and dry that it cracks in many places. Bullous eruptions which are most frequently noted about the knuckles are rare.

be due to a complicating disease such as tuberculosis. With the exception of the fever there may be in many cases of leprosy but little evidence of the general effect of the toxin of the leprosy bacillus. The neuritis and other nerve symptoms may be due to the effect of a circulating toxin since there are often comparatively few bacilli in the nerves and these are generally found in the nerve sheaths but on the other hand in nodular leprosy where there are usually much larger numbers of leprosy bacilli the nerves are often unaffected.

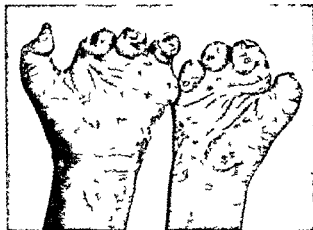


FIG 196.—Mutation of the hands. (After photo (Owald Cruz in t t t))

**Skin.**—The raised lesions of nodular leprosy tend to come out in numbers on the lobes of the ears over the eyebrows and on the cheeks as well as the backs of the hands and forearms and on the buttocks and feet. The soles of the feet and palms of the hands almost never show lepromata. In nodular leprosy the maculae are often single and flat and often appear on parts of the body covered by the clothing as the trunk and thighs or arms. The spots of leprosy are anesthetic often showing dissociation of sensation. The indurated macules of nodular leprosy then are succeeded by tubercled growths. A striking feature of all leprosy eruptions is that the hair falls out of the areas occupied by them. In the so-called tuberculo-eruptive leprosy the leprosy bacilli are present in the earlier stages and may later disappear. A number of observers including Fernandez and daRosa (1919) believe the tuberculo-eruptive reaction with intense tissue reaction is an allergic phenomena.

**Mucous Membranes.**—Pinkerton (1938) who has had 7 years of experience in the study and treatment of the pathological conditions of the nose, throat and larynx due to leprosy in Honolulu and Molokai believes that careful study of the mucous membrane will reveal that practically every leprosy patient has some nasal lesions due to leprosy. Rio (1936) found the nose affected in 81 per cent. In children epistaxis and nasal crusting occur most often as early symptoms. In the neural types the mucous membrane may be swollen, pale and dry during active progressions. Frequently there is freedom from pain on manipulation. Ulceration is not common in the primary neural types but it is common in nodular cases and in the late stages of neural types which have become complicated. The nodules in the mucosa often undergo ulceration. Perforation of the septum is frequently found and an ulcer of the septum is often the first place from which leprosy bacilli are discovered. The sinuses do not appear to be affected by the disease and while the cartilaginous septum is often involved and perforated, Murdock (1931) and Pinkerton have never found the nasal bone itself involved. There is remarkable freedom from symptoms of the disease in the pharynx until nodules are

of the finger and toe nails with the development of felons glazed skin or bullae. The latter, on rupturing are often followed by ulcerations. Absorption of the bones of the phalanges may also occur. Ulcers frequently form over the exposed portions of the hands and feet. They may penetrate and disorganize the joints and cause the fingers and toes to drop off, one after the other. The phalangeal bones may be completely absorbed and a distorted nail cap the end of the metacarpal bone (*lepra mutilans*) may result. Owing to the anaesthesia, lepers often burn or injure their fingers and toes. Perforating ulcers of the sole of the foot are more common in leprosy than in tabes.

Muscular palsies atrophies and contractures are more common in the face and upper extremity than in the lower extremity. We may have changes quite similar to those of progressive muscular atrophy, the thenar and hypothenar, as well as the interosseal muscles undergoing atrophy and resulting in claw hand, in this condition. There is extension of the first joint and flexion of the two distal joints of the fingers. Such hands may function quite well. Wrist drop is not uncommon, but foot drop is rare. Rarely the condition known as Charcot's joint may be observed. Vieira (1939) has studied the finger prints and found changes in 12 per cent of the neural and in 9 per cent of the macular neural. The changes are first found in the little and ring fingers pointing to ulnar disturbances.

Of the facial muscles the orbicularis palpebrarum is the most apt to show paralysis. Muscular atrophy may occur, so that the eyes cannot be closed, the upper lid may droop the lower lid becomes erected and the eye itself may become fixed. However the eyes are affected much less frequently in nerve leprosy than in the nodular form 45 per cent as against 85-90 per cent for nodular leprosy. The most common changes in nerve leprosy are ectropion of the lower lid and subsequent corneal ulceration with eventual loss of sight.

### SYMPTOMS IN DETAIL

**General Symptoms**—The general symptoms vary greatly. In some cases they may be strikingly few, while in others patients may lose flesh and strength rapidly.

**Temperature**—The temperature is often very variable. Sometimes there are daily rises in temperature without however the patient being aware of the fact. Muir has found a normal temperature continuing throughout the 24 hours in a few cases of leprosy. In the majority which have passed the primary stages an irregular temperature is observed falling to 96 F or 9 F in the morning and rising to 100 F or 100 F at night. In nodular leprosy the temperature curve is apt to be less regular than in the nerve form. More marked fever sometimes occurs at the onset of the attack and in addition during the course of the disease. Very frequently there is a febrile reaction which precedes or accompanies a new outbreak of skin lesions. This leptotic fever usually lasts for a few days or a week or so and then the temperature becomes afebrile. At such times sweating may be present and suggest malaria. Fever may also occur in the very advanced stages and particularly as a terminal event and this is often accompanied by chills and sweating and loss of flesh and sometimes by cough. At this stage of the disease it is often difficult to determine whether the temperature may not

## COMPLICATIONS

The most common complications of leprosy are tuberculosis and nephritis. Bronchopneumonia and cardiac lesions also occasionally occur. In a study of 360 autopsies in the Philippine Islands Pineda and Lara found the most frequent causes of death to be tuberculosis 24 per cent, nephritis 16.3 per cent, bronchopneumonia 9.3 per cent, dilatation of the heart 7.6 per cent, endocarditis 5 per cent, lobar pneumonia 3 per cent, and amoebiasis 3.3 per cent. Death was apparently due to leprosy itself in only about 2.3 per cent of the cases. Thus tuberculosis was by far the most prevalent complication, it not only having been responsible for 24 per cent of the deaths in the autopsied cases, but in 4 per cent of the total deaths at the leper colony during the same period. Rake, who performed 90 autopsies in the Trinidad leper asylum, also found about 30 per cent of the deaths to be due to tuberculosis, and in another series of 78 cases he found nephritis present in 29 per cent of the cases. At the leper colony in Panama the most frequent cause of death was likewise found to be pulmonary tuberculosis in 28 per cent, nephritis in 20 per cent, and pneumonia in 5.6 per cent. Syphilis may also complicate leprosy. In such cases of double infection it is not always easy to say except from the definite history which was the primary infection.

Vaccination with smallpox virus not infrequently causes the development of fresh leprosy lesions in lepers. So much so that in earlier years it was believed in Hawaii that vaccination might be a cause of exciting leprosy. Guillemin (1939) records reaction and the development of leprotic lesions following vaccination of 400 inmates of a leper institution in Africa. Vaccination in lepers sometimes runs an abnormal and violent course. On the other hand it has been asserted that an attack of smallpox has been followed by a subsidence or even by the temporary disappearance of the manifestations of leprosy. McCoy states that the same changes are reported to have sometimes followed successful vaccination with cowpox.

## DIAGNOSIS

**Clinical Diagnosis**—While in well advanced cases one who is accustomed to observe leprosy needs little aid in diagnosis, as the lesions are often strikingly characteristic, in early cases of the disease it must be recalled that owing to the slow progress of the affection there may be slight indications of it for long periods. The correct diagnosis of leprosy is obviously of much more importance than is the case with most diseases, since it is so likely to involve the whole future life of the patient. On the other hand, failure to diagnose a case may permit the exposure of many healthy individuals to infection. Therefore the greatest care must be exercised in diagnosing leprosy.

In arriving at a conclusion the following features should be particularly kept in mind:

formed. The nodules are found in the early stages at the base of the uvula and extend into the folds above the tonsils. More than 20 per cent of the leprosy patients have demonstrable infiltrated and nodular lesions of the tonsils. The nasopharynx frequently shares in the process by extension. Neural involvements of the tongue are not seen but nodules usually in the anterior third occur late in the disease. Leukoplakia is also quite common and the areas are often anaesthetic to tactile stimulus. Approximately 40 per cent of the cases with moderately advanced nodular lesions show leprosy lesions in some form in the larynx and in the great majority of cases the epiglottis is involved. The great majority of the more advanced cases present laryngeal lesions. The characteristic early symptom is the leprosy huskiness and peculiar vocal quality which strikes the experienced as suggestive. The patients complain of dryness and the tickling sensation that causes a dry unproductive cough. Not infrequently severe oedema of the larynx develops resembling that of some allergic phenomena. Laryngeal stridor and oedema of the glottis may occasionally result in fatal consequences. This acute reaction is also accompanied by high fever, chills, severe pain in the limbs and acute cutaneous lesions.

**Nervous System**—Besides the characteristic anaesthesia, various manifestations of neuritis are frequent, especially involving the ulnar, facial and peroneal nerves. The affected nerves show a fusiform enlargement and are tender. Later there may be trophic changes in the skin, bone and nails of the fingers and toes. Absorption of bone and perforating ulcers are frequent. Muscle palsies and atrophies, especially the main-*en griffe* are also common. The orbicularis palpebrarum is not infrequently paralyzed. The olfactory, optic and auditory nerves are rarely if ever involved. The reflexes are slightly exaggerated. Patients often complain of a sensation of cold. The temperature of anaesthetic fingers is sometimes reduced below normal. Some authorities have called attention to the frequency of a mental and moral apathy in leprosy and many lepers suffer from a marked mental apathy.

**The Circulatory System**—Honey considers a high pulse rate, especially in the morning, as characteristic of progressive stages of leprosy.

**The Blood**—The blood shows no characteristic changes but there is often a secondary anaemia. Leprosy bacilli are sometimes demonstrable in the blood, especially at the time of the febrile accessions. They are more common in the blood of the nodular cases. Anderson (1936) in a chemical study of the blood in Brazil, found that the total lipids are abnormally high in leprosy, the iodine index lower than normal in early leprosy and higher than normal in late leprosy cases that are not improved by treatment. The fatty acids were found to be increased in the later stages of the disease, but the cholesterol level in all stages was below the Brazilian normal average. Rubino found not only that the cholesterol content of the blood is reduced in leprosy but that the sedimentation rate is often decreased and generally speaking is greater in women than in men.

**The Eye**—In nodular leprosy, eye lesions, chiefly leprotic nodules in conjunctivae or iris with subsequent ulceration, are met with at some time in the course of the disease in almost 90 per cent of the cases. In nerve leprosy, corneal ulcerations, chiefly resulting from paralysis of the facial muscles with ectropion and other eye symptoms occur in about 45 per cent of the cases.

**Genito-urinary Symptoms**—Atrophy of the testicles, with increase of connective tissue, often results, but data would indicate that the procreative power of the female is but little diminished. Lepers often die of renal complications, the kidney lesions being rather those of amyloid change. Bacilli may be eliminated in the urine during accessions of fever. Radna (1939) emphasizes the elimination of leprosy bacilli in the urine after the administration of arsenic in 9 cases in which the urine was negative before treatment with novarsenobenzol. Anderson has found that the nephritis which may complicate leprosy is aggravated by treatment with chaulmoogra oil, metals, dyes and especially methylene blue. In his opinion, patients with damaged kidneys should not be given intensive specific treatment with renotropic agents.

**The Lymphatic Glands**—These tend to enlarge and show bacilli, but rarely suppurate. The inguinal, cervical and epitrochlear glands are most often enlarged.

eyebrows palsy of the orbicularis palpebrarum and nodular thickening of the lobes of the ears are all characteristic of the disease. However there is no single symptom that can be regarded even as generally the earliest one.

Muir in the study of nearly a thousand cases in Indian asylums with reference to the earliest noticeable lesion found that there was a far greater prevalence of the lesions radiating from the nose as a center which probably resulted from nasal infection spreading through the lymphatics of the face. Second there was a great excess of lesions upon the extensor surfaces this being due he thought because they were more exposed than the flexor surfaces. Third a large number of lesions were situated on the feet especially the soles all this indicating direct infection through the skin of areas uncovered by clothing.

In many cases however the appearance of an anaesthetic spot is frequently the first symptom. Gomez (1924) in studying one hundred cases of leprosy with special reference to the initial lesion found that the great majority of the patients declared that they first noticed numbness of the lower extremities usually more or less localized to a well defined area. Next in frequency was the appearance of red spots usually on the face and most frequently on the cheek about the malar bones. In a few of the cases the spots were single but in the majority they were either multiple or generalized. Next in frequency were paler or whitish maculae occurring also more frequently on the face. In a few cases they were single but more frequently multiple or generalized. In a few cases the first symptom noticed was the appearance of nodules usually generalized in one case in the ear only. The author emphasized that the great frequency of nervous manifestations is remarkable and that it is impossible to point out with certainty any particular lesion as the initial one it being probable that the microbe enters the body in many cases without producing external change multiplying in the lymph spaces and spreading in the body.

**Differential Diagnosis**—Leprosy may be confused with a number of cutaneous diseases and particularly with the cutaneous lesions of syphilis tuberculosis and naso oral leishmaniasis. Syringomyelia also may cause confusion. In India it is stated that the disease most frequently mistaken for leprosy is probably syphilis. Some of the skin lesions in syphilis do resemble those of both nodular and nerve leprosy. Muir states that he has seen what looked like typical leontiasis of the face which is frequent in leprosy but the case turned out to be one of syphilis. The most important points in the differentiation of the two diseases are the presence of anaesthesia in nerve leprosy and its absence in syphilis the history of the manner of contraction of the disease in syphilis and the effects of arsphenamin or other arsenical preparations or of mercury in syphilis. However the leprosy condition may recede following the administration of arsenicals though usually more slowly than in syphilis. Finally a careful bacteriological examination should be made for the discovery of leprosy bacilli or spirochaetes in the lesions.



With reference to the history the habitation or community in which the individual has resided and possible exposure or contact with persons affected may excite suspicion. The mucous membranes of the nose are sometimes infected and particularly in the nodular form and sometimes in the nervous one epistaxis is occasionally the first sign of the disease. Later the flattened nose of the leper is often very striking resulting as it does from the disappearance of the septum through ulceration. Still points out that one should always examine the lobes of the ears or region of the eyebrows and feel for shot like nodules. One should also particularly seek for areas of discoloration upon the skin with anaesthesia for symmetrical eruptions of macular areas with bilateral distribution which



FIG 197.—Leprosy bacilli in the coarsum. (From report of Harvard African Expedition in Liberia and the Belgian Congo.)

may be dusky red or fawn colored and elliptical in shape the periphery of the lesion being elevated and more deeply pigmented while the center remains lighter in color. Such lesions should be particularly sought for on the buttocks, legs and forearms and should be carefully tested for anaesthesia. The occurrence of trophic disturbances is of particular importance such as perforating ulcers muscular atrophy especially of the hands resulting in the condition known as claw hand. Other deformities of the hands and feet resulting from loss of phalanges and persistent ulcers at the articulations of the phalanges of the fingers and toes are particularly suggestive. Facial paralysis is also a very frequent condition in leprosy. The occurrence of typical nodules and their distribution is often pathognomonic of the disease. The early involvement of the nasal pharynx and the larynx and the formation of lesions in the vocal cords resulting in a peculiar resonance of the speech is also particularly characteristic of leprosy. Finally in the advanced cases the expression of the face leonitiasis satyrasis ectropion of the eyelids and of the lips the loss of the

fluid and the Wassermann reaction with it has been reported as negative Kolmer has claimed that non syphilitic lepers give a negative serum reaction with his modification of the complement fixation test but others using this technique have obtained approximately the same number of positive reactions as by the usual method

*Tuberculi* s s.—Lupus vulgaris may not infrequently resemble leprosy especially when the nose or mouth is affected. However the apple jelly color distribution size of the lesions consistency and method of development of the lesions in lupus are usually sufficiently distinctive. In leprosy the presence of anaesthesia will also aid in the differentiation. Unfortunately the tuberculin test is usually of no assistance in differentiating these two diseases. Goodpasture obtained 100 per cent positive complement fixation test in 24 cases of nodular mixed and anaesthetic leprosy using an antigen composed of a suspension of *Bacillus tuberculi* s s of human origin. The discovery of the tubercle bacillus on the one hand or of the leprosy bacillus on the other will obviously give a definite decision. However for differentiation of these two microorganisms unless the leprosy bacilli are present in large numbers within endothelial cells inoculation of a guinea pig with some of the suspected material may be necessary. A negative result will usually exclude tuberculosis.

*Leprosy of Leishmaniasis*—In certain parts of the tropics particularly in parts of South America leprosy is frequently confused with naso-oral leishmaniasis. The early destruction of the nasal septum in leprosy and the flattening of the nose and necrosis of the alae nasi may be very confusing particularly in relation to the late stages of leishmaniasis. Nasal scrapings however will usually reveal the leprosy bacillus. Unfortunately in the late stages of naso-oral leishmaniasis the leishmania are found only in very small numbers or may be absent.

*Syringomyelia or Syringomyeloid Disease*—Still another that is particularly the most difficult disease to differentiate from leprosy is syringomyelia. The symptoms of this disease are produced through a glomatous new growth about the central canal of the spinal cord with cavity formation due to the development of embryonal neuroglia tissue in which hemorrhages or degenerations take place with the formation of cavities. It is characterized by neuralgic pains cutaneous anaesthesia and painful destructive whitlows with loss of tissue and hence may be sometimes considerably confusing in diagnosis. In syringomyelia the dissociation is as marked as with leprosy. In syringomyelia however the upper extremities are as a rule alone affected and the muscular atrophy is more of the scapulohumeral type with involvement of the trunk muscles causing scoliosis rather than of the thenar and hypotenar eminences that while the fingers may be more contracted and rigid than in leprosy the *manus en griffe* is not produced. The anaesthetic areas of syringomyelia continue to sweat and the reflexes may be also spastic symptoms and speech defects in syringomyelia but even so the differential diagnosis may be sometimes specially difficult. Clinico records a case illustrating the difficulty of establishing a diagnosis where many symptoms of both leprosy and syringomyelia are present. In a case which he reported of definite speech dysphagia Kombergism spastic and toxic gait head bent forward with limited movement lordosis at the mid-dorsal spine atrophy of the *ternocleidomastoidei* muscles nystagmus and anaesthesia over the back of the phalanges and palms of the hands and over the front of the thighs legs and feet were all present. The fingers of both hands were greatly deformed with atrophy of the thenar muscles. There was a tuberculous eruption upon the chest. The author concluded that nystagmus difficulty in swallowing husky voice impotence negative Wassermann and absence of nasal leprosy bacilli were in favor of a diagnosis of syringomyelia in which there was an unusual type of deformity resulting in a condition simulating the claw hand. Undoubtedly the bacteriological examination and the finding of the leprosy bacilli is the most satisfactory means of the differentiation of these two diseases.

*Raynaud's Disease* may be particularly differentiated by the progressive ulcerative process limited to the extremities involved and by the absence of nerve trunk involvement.

**Wassermann Reaction**—Unfortunately the Wassermann reaction is very frequently of no assistance in the differentiation of syphilis and leprosy

Cooke (1923) tabulated 1397 cases of leprosy in which up to the time of his publication the Wassermann reaction had been performed. In 50 per cent of the cases a positive Wassermann reaction was obtained. 723 cases were of the nodular and mixed variety and in this group 60 per cent were positive. 405 were purely anaesthetic cases and only 25 per cent of these gave positive reactions. Later Goodpasture (1923) found that the Wassermann reaction was positive in 60 per cent of untreated nodular and mixed cases of leprosy and in 84 per cent of similar cases treated with chaulmoogra oil or its products. Lloyd (1924) in a study of 228 adult lepers found a positive Wassermann reaction in 41 per cent of all cases, in 27 per cent of anaesthetic cases, in 47 per cent of mixed cases and in 63 per cent of nodular cases. In a series of 58 leper children he found a positive Wassermann reaction in 62 per cent, in 47 per cent of anaesthetic cases, in 80 per cent of mixed cases and in 100 per cent of nodular cases, thus much higher figures were obtained in children than in adults. Leao also examined the Wassermann reaction in leprosy. He obtained a positive reaction in macular cases of 87 per cent, in tubercular cases in 65 per cent and in the nervous form of 27 per cent. He also examined the Sachs Georgi test in leprosy and found a positive reaction in the macular form in 62 per cent, in the tubercular form in 39 per cent and in the nervous form in 17 per cent. McKinley and Soule (1935) performed the Kolmer Wassermann and Kahn reactions with the sera of Philippine patients having a severe lepra reaction but presenting no evidences of syphilis or yaws. These reactions were positive in 35 and 33 per cent. Finally Nicolau and Banciu obtained a positive Wassermann reaction in 22 out of 27 lepers. However on using the method of progressive dilutions with physiological salt solution in cases of leprosy and syphilis giving positive reactions they found that the titers for the reaction in leprosy greatly surpassed those of syphilis. They considered that this method therefore may be of value in the diagnosis of leprosy. However from what has been said it is obvious that in general the reaction is of little service in differentiating these two diseases.

Maltaner (1940) by the employment of quantitative methods of complement fixation found that in the examination of 47 specimens of sera from leprosy cases that only 3 reacted to high degree while 5 others reacted to a less marked degree and 2 gave slight reactions. The remaining 35 were negative. In contrast a large proportion of the leprosy sera reacted to the tubercle antigen.

Some authorities upon syphilis believe that a positive Wassermann reaction in leprosy may be accounted for by concomitant infection with syphilis or yaws. However in many cases of leprosy with positive Wassermann reaction no evidence of either syphilis or yaws has been found even at autopsy. McCoy has pointed out that the percentage of positive reactions in the United States is also high and that in these cases yaws can be excluded. It would appear that in many cases of leprosy there are serological changes analogous to those occurring in syphilis and yaws. The strength of the reaction fluctuates during the course of the disease and is most often positive in the febrile exacerbations. Pinkerton (1938) has observed negative Wassermann reactions on lepers on entrance and has observed that as the leprosy disease progresses the Wassermann and Kahn precipitation tests become positive and the intensity of the test diminishes as the leprosy recedes.

Antisyphilitic therapy has not been of value in the absence of syphilitic lesions and some workers have felt that it was actually harmful. In non syphilitic lepers no changes have been found in the cerebrospinal

The organism owing to its peculiar structure is somewhat difficult to stain and a powerful dye such as carbolfuchsin is often necessary to demonstrate it satisfactorily. The leprosy bacilli usually take up basic stains some but more easily but also decolorize more readily than tubercle bacilli. However there are certain variations in this respect with organisms obtained from different sources. Leprosy bacilli may sometimes be overlooked in sections owing to the readiness with which the stain is extracted from the bacilli in dehydrating and clearing the sections. If leprosy is readily stained by Gram's method. With the Ziehl-Neelsen-Gabbet carbolfuchsin solution as used for the staining of the tubercle bacillus most satisfactory results are obtained. For the staining of the organism in section his method or the Gram-Weigert stain or fuchsin-methylene blue stain of Baumgarten all give very satisfactory results. The Baumgarten stain does not color the tubercle bacillus so readily.

In from 50 to 80 per cent of leprosy cases the mucous membrane is found to be infected. Muir points out the bacillus has been found in some cases in the nasal mucus where at the time there were no other signs of leprosy.

On the other hand the examination of the nasal mucus often gives negative results and sometimes confusing ones. When the leprosy bacilli are found in great abundance and in masses within cells there is little doubt about the diagnosis but sometimes in the nasal mucus the leprosy bacilli are scanty or are so atypical in appearance that one may be left in doubt as to the nature of the acid fast organism. Where only a few isolated acid fast bacilli are found it must be borne in mind that there are other acid fast bacilli besides the leprosy bacillus which might occasionally be found in the nostrils. In Manila acid fast bacilli were found in tap water. Morgan who has made examinations of the nasal mucus obtained by means of sterile swabs composed of cotton wool wrapped around the end of an iron wire found that although 29 of his cases were diagnosed as leprosy on other ground leprosy bacilli were found in the nasal mucus in only two of them. The organisms are more apt to be found when coryza with mucous exudate is present. In order to excite a drug coryza the standard procedure has been to administer 65 gr. of iodide of potash after which the leprosy bacilli may frequently be found in the secretion produced. Though a better method is to scrape with a scalpel the mucous membrane it should be borne in mind that even this does not always give a positive result in leprosy cases.

In some cases particularly of the nerve form it may be necessary to remove a small bit of tissue. Film preparations should be made by smearing the cut surface of a portion of this tissue over the clean glass slide while another portion of the tissue may be hardened in alcohol or Zenker's solution and subsequently stained as already described. Usually as many leprosy bacilli are found in the film preparations made from the tissue as in the section itself but the section will obviously reveal the pathological histology which may in some instances be of further assistance in diagnosis.

Kobayashi recommends particularly for the early diagnosis of leprosy the puncture and examination of fluid from the testes. He has found that in numerous cases of leprosy he could demonstrate the bacillus from such fluid even from cases having no outward changes in the testes and even in cases where the bacteriological examination of other material had proved negative. In this connection it is interesting to recall that Morisseau (1914) found that the negroes of the upper Volta region in West Africa readily recognize the early stages of leprosy and look particularly for discolored patches on the body pains on pressure over the distal phalanges and a nodule in the testicle. In choosing captives they always avoid any showing these signs.

Certain other skin diseases may momentarily cause confusion. *Ringworm* is some times closely simulated by some skin lesions of leprosy particularly when they are red and scaly. Microscopical examination however will reveal the diagnosis leprosy bacilli being found in one instance or the characteristic trichophyton in the case of ringworm. *Tinea versicolor* may be mistaken for leprosy particularly when the lesions occur on the face where they are apt to be a little lighter than upon the chest. The writer on two occasions has found boys with tinea versicolor infection upon the face in leper colonies or asylums. Here again microscopical examination will immediately differentiate the infections the presence of *Microsporum furfur* being easily found in scrapings from the patches.

*Yaws* could hardly be confounded with leprosy except in some of its tertiary manifestations. Yaws also immediately yields to injections of arsphenamin and similar arsenical preparations while leprosy is not affected promptly or at all by such treatment.

*Gangosa* may in some instances be confused with advanced cases of leprosy. The chief diagnostic difference in this affection would be the absence of the leprosy bacillus. From the clinical appearance at times the differentiation might be impossible. Murdock (1931) and Pinkerton (1938) found no instance of involvement of the nasal bone in leprosy.

*Fibroma molluscum* which is not uncommon in India and other parts of the Far East might occasionally cause confusion. Here again the absence of the leprosy bacilli will decide the nature of the affection.

Archibald has recently reported from the Sudan a case of leprosy which might have been confused with *molluscum contagiosum*. This case from the lesions might well have been termed military leprosy and sections of the lesions revealed large numbers of leprosy bacilli.

*Leucoderma* sometimes called white leprosy by the natives bears a certain resemblance to the pale macular patches of leprosy which have already been discussed. However the abrupt margins and absence of anaesthesia in leucoderma are quite distinctive. Another noticeable difference in the lesions is that the leprosy patches rarely perspire. A hypodermic injection of pilocarpin may be given in order to differentiate this point.

**Bacteriological Diagnosis**—In some instances the practical diagnosis of leprosy can be made before a positive bacteriological result is obtainable and this is especially true in certain neural cases of the disease. However the diagnosis of leprosy should if possible always be confirmed even if it is not made by the microscopical examination and detection of *M. leprae* in the lesions. Microscopical preparations should be made from the macules, papules or nodules which may be present on the skin.

The skin should first be thoroughly scrubbed with soap and water and then with alcohol and ether to free it from any saprophytic acid fast micro organisms that might be present on the surface. The lesion should then be grasped by the thumb and index finger and pressure applied until the overlying skin becomes anaemic. With a scalpel a very small incision should then be made through the epidermis and well into the corium and the surface scraped lightly. This causes a small amount of serous exudate to appear which should be spread upon glass slides. The specimens should be obtained without a large amount of blood since blood renders the examination less satisfactory. Scrapings should also be made from the nasal cavities. A narrow bladed knife should be employed and the mucous membrane covering the cartilaginous portion of the septum scraped and the material on the knife then spread on a clean slide. Scrapings should also be made of any lesions in the nostrils such as nodules or ulcers. The knife should of course subsequently be thoroughly sterilized. All these preparations should then be hardened either by heat or with absolute methyl alcohol and stained by the Ziehl Neelsen method (decolorizing lightly with 3 per cent HCl in alcohol) or 5 or 20 per cent H<sub>2</sub>SO<sub>4</sub> in water. Specimens may be stained with hematoxylin to obtain the histological background before the acid fast stain is applied.

earlier exist but it seems to have been established that the reaction may occur in leprosy quite independently of the existence of syphilis and that in leprosy complement fixing bodies are developed to some extent which are similar to those found in the serum of cases of syphilis. This obviously is only another demonstration that the reaction as generally performed is not a specific one.

The *tuberculin* reaction is given also by cases of leprosy and Good pasture has employed the complement fixation test in which an antigen composed of a suspension of *Bacillus tuberculosis* was employed as a means of measuring the response of leprosy patients to treatment with chaulmoogra oils the reaction showing a tendency to become negative in cases in which leprosy bacilli were no longer demonstrable.

Taylor and Malone employed Dryer's method of defatting tubercle bacilli by the application of formalin and acetone to the leprosy bacilli. A fine gray powder was thus obtained which may be kept well in a desiccator. The sera of 100 lepers in the Rangoon leper asylum were tested with suspensions of this antigen and all 37 of the nodular leprosy cases all but 2 to 50 nerve cases and 12 of 13 of the mixed cases gave positive complement fixation results making 97 per cent of the total cases positive. The great majority of the leper asylum cases had had the disease for 3 years and longer. They tested the sera of 30 cases of tuberculosis but obtained positive reactions in 20 per cent only.

With reference to the contradictory results which have been obtained with regard to the complement fixation reaction as applied to leprosy Lewis and Aronson tested 45 sera from 39 lepers using as antigen various acid fast bacilli including those believed to have been cultivated from lepers and *Bacillus tuberculosis*. All gave numerous positive results varying between 81.8 and 96.8 per cent. None of the control sera gave any positive results with Clegg's organism. The authors however point out that while the reaction may be of practical value in diagnosis these reactions are no evidence of any of these bacilli being the cause of leprosy since most frequent reactions were obtained with the tubercle bacillus.

Black (1939) has performed the complement fixation reaction with the highly chromogenic strongly acid fast bacillus which Lleras (1936) cultivated from the blood of cutaneous cases of leprosy. Lleras reported positive complement fixation in 99.1 per cent of cases of leprosy. Black using this same organism obtained a positive reaction in 94.5 per cent of cases of leprosy bacteriologically positive, a positive reaction in 37.5 per cent of cases of leprosy bacteriologically negative and a positive reaction in 0.3 per cent of 329 sera of miscellaneous cases (not leprosy). A positive reaction was also obtained in 6 per cent of 50 cases of tuberculosis. Pereira (1938) also with this organism obtained a positive reaction in 14 per cent of 50 healthy persons. Lowe (1939) in Calcutta studied the complement fixation test in leprosy using antigens from 6 different acid fast bacteria. These antigens were prepared from the so-called lepra bacilli of Kedrowski, Lleras, Bay and Duval. An antigen from the tubercle bacillus was also included. The tests were performed on 123 cases of leprosy and in 60 cases of other diseases. All the antigens behaved more or less similarly though the one prepared from Lleras' organism gave a higher number of positive results than the other antigens. The greater sensitivity of Lleras' antigen however did not seem to depend on any specificity as a positive reaction was observed in non leprosy cases also.

Attention has already been called to the fact that leprosy bacilli may sometimes be found in the blood of nodular cases, and especially at the time of the febrile accessions. However a negative result should not exclude the diagnosis of leprosy.

For finding leprosy bacilli in the blood from 5 to 10 cubic centimeters should be removed from a vein with a syringe containing a small amount of 1 per cent sodium citrate solution. After centrifuging the sediment should be treated with 10 per cent antiformin at 37 C for one hour. The mixture should then be again centrifuged and after washing to get rid of the antiformin the sediment is spread out upon a slide and stained in the usual manner. Smith and Rivas added 10 volumes of 2 per cent acetic acid and 1 volume of blood centrifuge and then make film preparations from the sediment. In nerve leprosy examination of the blood is of little use as organisms are rarely present.

In leprosy as in other infectious diseases the rate of sedimentation of the red blood cells may be increased. Although the test has little diagnostic value it may be an index of the progress of the disease. At times it may be diminished.

Rubino has shown that the serum of lepers causes agglutination and sedimentation of formalized sheep red blood cells within an hour. Confusing hetero agglutinins in the sera are removed previously with non formalized cells. The reaction appears to be specific but it is not very sensitive. Since it is positive only in the well marked cases its usefulness as a diagnostic procedure is limited.

If enlarged glands are present as for example in the epitrochlear region gland puncture or excision of the gland and examination as already described for tissues may reveal promptly the diagnosis.

The only other acid fast bacilli which are likely to be confused with *M. leprae* are the *Bacillus tuberculosis* and the *Bacillus smegmae*. Tubercle bacilli are found in lesions in much smaller numbers as a rule than leprosy bacilli. However, as already pointed out in some lesions leprosy bacilli may be scanty. The differences in the pathological histology of leprosy and tuberculosis which have already been referred to will usually aid in their differentiation.

Inoculation of a guinea pig with the suspected material will give a negative result in the case of the leprosy bacillus and a positive one usually in the case of the tubercle bacillus. The smegma bacillus also is usually not found in such large numbers as the leprosy bacillus and not within endothelial cells. While the differentiation of these bacilli has been suggested by means of their acid fast properties and permeability of stains such methods are really unreliable at times and of little value in accurate differentiation of them. When the leprosy bacillus occurs only in small numbers as it sometimes does and is not intracellular it must be remembered that it has no single characteristic that will differentiate it from *Bacillus tuberculosis* or *Bacillus smegmae*.

The Roentgen rays have sometimes been utilized in the recognition of very early atrophic changes in the bone where commencing absorption of the phalanges may occur. In some cases there is even disappearance of the terminal phalanx of some of the toes. This method may be of considerable value particularly if there are no other satisfactory indications of leprosy to suggest a bacteriological examination.

**Serological Tests**—A large number of investigations have been made with the hope of discovering satisfactory serological tests of value in diagnosis. Reference has already been made to the fact that some 40 to 50 per cent of the cases of leprosy have given a positive Wassermann reaction. There was formerly considerable discussion as to whether this reaction could be obtained in leprosy in cases in which syphilitic infection did not

earlier exist but it seems to have been established that the reaction may occur in leprosy quite independently of the existence of syphilis and that in leprosy complement fixing bodies are developed to some extent which are similar to those found in the serum of cases of syphilis. This obviously is only another demonstration that the reaction as generally performed is not a specific one.

The tuberculin reaction is given also by cases of leprosy and Good pasture has employed the complement fixation test in which an antigen composed of a suspension of *Bacillus tuberculosis* was employed as a means of measuring the response of leprosy patients to treatment with chaulmoogra oils the reaction showing a tendency to become negative in cases in which leprosy bacilli were no longer demonstrable.

Taylor and Malone employed Dryer's method of defatting tubercle bacilli by the application of formalin and acetone to the leprosy bacilli. A fine gray powder was thus obtained which may be kept well in a desiccator. The sera of 100 lepers in the Rangoon leper asylum were tested with suspensions of this antigen and all 37 of the nodular leprosy cases all but 2 to 50 nerve cases and 12 of 13 of the mixed cases gave positive complement fixation results making 97 per cent of the total cases positive. The great majority of the leper asylum cases had had the disease for 3 years and longer. They tested the sera of 30 cases of tuberculosis but obtained positive reactions in 20 per cent only.

With reference to the contradictory results which have been obtained with regard to the complement fixation reaction as applied to leprosy Lewis and Aronson tested 45 sera from 39 lepers using as antigen various acid fast bacilli including those believed to have been cultivated from lepers and *Bacillus tuberculosis*. All gave numerous positive results varying between 81.8 and 96.8 per cent. None of the control sera gave any positive results with Clegg's organism. The authors however point out that while the reaction may be of practical value in diagnosis these reactions are no evidence of any of these bacilli being the cause of leprosy since most frequent reactions were obtained with the tubercle bacillus.

Black (1939) has performed the complement fixation reaction with the highly chromogenic strongly acid fast bacillus which Lleras (1936) cultivated from the blood of cutaneous cases of leprosy. Lie as reported positive complement fixation in 99 per cent of cases of leprosy. Black using this same organism obtained a positive reaction in 94.5 per cent of cases of leprosy bacteriologically positive a positive reaction in 37.5 per cent of cases of leprosy bacteriologically negative and a positive reaction in 10.3 per cent of 329 sera of miscellaneous cases (not leprosy). A positive reaction was also obtained in 6 per cent of 5 cases of tuberculosis. Perczka (1938) also with this organism obtained a positive reaction in 4 per cent of 50 healthy persons. Lowe (1939) in Calcutta studied the complement fixation test in leprosy using antigen from 6 different acid fast bacteria. These antigens were prepared from the so-called lepra bacilli of Kedrow ski, Lleras, Bayon and Duval. An antigen from the tubercle bacillus was also included. The tests were performed on 13 cases of leprosy and in 60 cases of other diseases. All the antigens behaved more or less similarly though the one prepared from Lleras' organism gave a higher number of positive results than the other antigens. The greater sensitivity of Lleras' antigen however did not seem to depend on any specificity as a positive reaction was observed in non leprosy cases also.



McKinley (1938) performed over 5000 intradermal skin tests with antigens prepared from various acid fast bacteria, some of which had been isolated from cases of leprosy by McKinley and Soule. The antigens included the TPT (protein) of many of these organisms, the protein polysaccharide phosphatide leprosin (wax) and leprosinic acid from one strain of acid fast isolated from a case of leprosy and a protein prepared from the fibrin of the blood from leprosy cases. Cases of leprosy in various stages of the disease, individuals who had been in contact with the disease, suitable control individuals having had no contact with leprosy, individuals of both sexes and varying ages, cases of both neural and cutaneous leprosy predominating and cases bacteriologically positive and negative have been given intradermal tests with these antigens.

In no instance did the positive reactions in leprosy exceed the negative and no definite conclusions therefore can be drawn. The study indicated that in none of the antigens studied have we found a specific antigen for a diagnostic skin test for leprosy. The work also suggests that the supposed strains of *M. leprae* from which several of the antigens were prepared are not related specifically and etiologically to the disease.

**Rubino's Reaction**—A number of reports have appeared recently in the literature regarding the value of this reaction in the diagnosis of leprosy. The method of Westergren has been particularly used. The citrated blood is placed in a tube and the rate of the sedimentation noted in millimeters at the end of 1 to 2 to 24 hours. In general the rate has been greater in women than in men. In leprosy the sedimentation rate may be decreased below that of normal subjects and at the same time the cholesterol content of the blood has been found reduced. Rubino more recently advocated an agglutination sedimentation test with formalized sheep's corpuscles. The sensitiveness of the reaction is reported to be clear and not obtained in any condition except leprosy; the serum of the lepers containing a specific substance which causes rapid agglutination and sedimentation of formalized sheep's corpuscles. The reaction is regarded positive if the sedimentation is produced in less than an hour with the formalized corpuscles.

Imbert (1936) has studied the reaction in Puerto Rico, employing different dilutions of the serum, fresh corpuscles with formol and non formalized corpuscles as control. The reaction was positive in 70 per cent of all cases of leprosy, 86 per cent in the nodular cases, 70 per cent in the mixed and 62 per cent in the nervous types. No positive reaction was found in the non leprosy cases.

## PROGNOSIS

The prognosis in leprosy is unfavorable although spontaneous improvement frequently occurs at different periods and the disease has a tendency towards self healing. In a number of cases in which the lesions seem to be especially confined to the nerves the disease seems to die out.

The Leprosy Commission of the Philippines in 1935 came to the conclusion that the disease must be considered incurable. The incidence of relapses in the cases around Manila was 46 per cent and in Cebu 39.1 per cent. Postmortem examinations made at the Culion leper settlement of individuals who succumbed from other causes showed that *M. leprae* was present in the nerves of almost all the cases which had been supposed to be cured. Postmortem examinations also showed that in cases in which during life the palms of the hands and soles of the feet

and other areas of skin appeared normal clinically many leprosy bacilli were found

The Commission pointed out that the treatment of children of lepers does not prevent them from becoming bacteriologically positive lepers that the pathologic processes which develop in the skin of children of lepers is similar or identical to those in the skin of the cured lepers and this shows clearly the incurability of the disease because if the drug fails to prevent the children of lepers from becoming infected and with bacilli present it is clear that the same drug cannot prevent a leper supposedly cured from developing a relapse of the disease if he lives long enough

This Commission however believes that many lepers may be freed of the demonstrable presence of leprosy bacilli by chaulmoogra treatment the surface of the body becoming free from the causative organism

During the past 10 years about 3500 patients have been discharged from the Cebu leper asylum as bacteriologically negative However it is known that the disease has recurred in about half of the discharged cases Burgess (1938) points out that apparently the relapse of some cases at least is not due solely to the fact that those paroled from leper colonies go back into the same conditions of living as those from which they came Very recently there was conducted at Cebu P I an interesting experiment along these lines Five young men paroled from the Cebu leprosanium were taken into the home of a Catholic priest as house boys This was done for the purpose of seeing whether sanitary living conditions and proper diet would prevent relapse All these became positive again within a period of from 4 months to 4 years except one This latter after a two year period is still quiescent but this was practically a burned out case when paroled

As a rule nodular leprosy runs its course more quickly than pure nerve leprosy and in nodular leprosy intercurrent infections are frequently the cause of death Tuberculosis results fatally in about 23 per cent of the cases and nephritis in almost 30 per cent a combination of tuberculosis and renal disease occurring in about 10 per cent Lepers especially those with nerve leprosy may live for 20 to 40 years

#### PROPHYLAXIS

Segregation is the most important prophylactic measure Leprosy tends to spread where there is marked personal uncleanness and close contact with lepers in overcrowded quarters

With reference to disinfection many authorities consider the free use of soap and water the most important means of avoiding the infection Rooms or buildings formerly occupied by lepers and which are to be used for the dwelling of others should first be fumigated in order to destroy any insects present which may possibly assume a role in the occasional transmission of the disease Later there should be a general disinfection of the room or house with bichlorid solution 1:1000 or carbolic acid 1:30 and all personal belongings dishes etc should be disinfected either with one of these solutions or in boiling water

As noted under epidemiology there seems to be little evidence to show that insects play any part in the transmission of leprosy. Nevertheless it would seem advisable to prevent flies from becoming contaminated with the discharges from leprosy ulcerations which so often teem with leprosy bacilli. This possible method of rare mechanical transmission by flies of bacilli to other individuals would seem more deserving of attention than the question of the possibility of the taking up of bacilli from the blood by mosquitoes bedbugs or biting flies and their transference to man. While the leprosy bacilli are found in the blood of patients with nodular leprosy chiefly during the febrile accessions in all of the ordinary insects the bacilli seem to disappear in a very short time except in a few instances in the tick. However acid fast bacilli have been reported to subsist for a longer time in the cockroach. These pests can generally be easily destroyed by sprinkling the places they inhabit with a little sodium fluoride.

**Segregation**—The leprosy individual constitutes a source of danger to the community in which he lives. He is the only source from which infection of another individual may result. The only effectual way of suppressing the disease in a community is through the detection and isolation of all existing lepers. The prevention of their contact with normal persons especially with children is a most important factor. Segregation has been generally considered as the only means of eradicating leprosy. While it is generally considered the one proven prophylactic measure there are still those who question its practical value.

One of the best recognized efforts at compulsory segregation has been carried out in Hawaii but there has not been any marked influence on the spread of the disease among the native Hawaiians. However, in 70 years of segregation the incidence rate has been somewhat reduced as well as the total number of known cases. Nevertheless, from 50 to 100 new cases have been reported in Hawaii each year. In recent years better education and better general living conditions have apparently helped in reducing the amount of disease.

In Louisiana compulsory institutional segregation was begun in 1894 when the Louisiana leper home was established near the present institution at Carville. For the 42 years of its existence and the 19 of the national leprosarium the average annual number of new cases admitted from Louisiana has been approximately 12 though for the last 4½ years it has been 9. Hasseltine (1938) points out that the manner of enforcement in different years of the law of compulsory segregation has an influence on the number of new cases discovered. Some of the patients become dissatisfied and run away again constituting a focus of infection to the community. Since 1921 there have been 250 absconders. Of this number, 160 returned nearly one half coming back voluntarily. Eight are known to have died and 82 are still at large though some of these probably are dead. To lessen absconding a system of permits to visit their homes has been established.

Burgess (1935) emphasizes that after 30 years of heroic segregation of the cases in the Philippine Islands we are faced with the knowledge that there is no striking evidence that the number of clinical observable lepers has been decreased.

Nevertheless, at the International Congress of Leprosy (1938) the representatives of most countries considered segregation as a necessary

measure. The representatives from Norway, Sweden, Iceland and Finland related the diminution of leprosy in those countries in the first three of them by legal segregation and in Finland with voluntary hospitalization. In Norway the reduction has been from 2850 in 1856 to 18 in 1937, in Iceland from over 100 in 1900 to 32 in 1937, in Finland from 93 to 19 since 1904. In Sweden there were at the time of the report only 10 cases recognized.

However, in some localities segregation is regarded as impracticable. McCoy (1938) believes that in the areas in which infection appears to be rather readily communicated, lepers should be isolated in order that we may take advantage of whatever measure of success attends isolation. He personally doubts that the success is very considerable. In the areas in which experience has shown that the disease does not spread, there would probably be no necessity for isolation of cases, save from the standpoint of public charity or from the sensibilities of the community. In the matter of public health control, the question should be settled by the individual case. Cases judged to be highly infective obviously should be segregated. This indicates particularly those of the nodular or mixed type of the disease. Purely neural cases may be allowed liberty.

Rodriguez (1938) summarizes the leprosy activities of the Bureau of Health of the Philippines under 3 main heads: (1) the control of open cases by isolation and treatment in leprosaria; (2) discovery and treatment of closed cases (not infectious) by means of skin dispensaries conducted separately from the leprosaria; (3) the following up and treatment of paroled cases of leprosy that have become infective. After 10 years experience it has been found that these so-called skin dispensaries are the best means of discovering and attracting leprosy cases. In the Philippines, in Cebu only about half the cases of leprosy have been found to have contracted the disease in houses occupied by other lepers.

Where a leper is not excreting bacilli or where acid fast organisms cannot be found after careful search, there may be generally little danger of contagion. Such patients, however, should report for examination every few months. Evidence as to contact indicates that all young children are particularly liable to the infection. This has been noted not only for children of lepers but also for brothers and sisters of lepers. Even if segregation of lepers is not carried out as regards adults, it should be for children and infants and young children should be separated at birth from their leper parents or parent. Cochrane (1939) who made an intensive study of child leprosy in India, believes there is considerable evidence indicating that the more serious type of leprosy develops where contact is maximal and that the most important form of contact is intra-familial. *Home infections in different regions vary from 25 per cent to 75 per cent of the total cases.* Rodriguez (1938), Koeston (1938) in Japan found a parallelism between the chances of contact with lepers in the community and the frequency of the disease.

A remarkable feature in connection with leprosy is the hysterical dread that many communities still have of a leper notwithstanding the

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in the baths. It seems improbable however that any medicament employed in the bath has any special therapeutic property.

The leper being generally looked upon as an outcast from society and usually shunned by most people is often apt to have fear of the discovery of his condition and after his isolation to brood upon it. Sometimes he assumes a hopeless attitude regarding his cure. As a result he often becomes exceedingly mentally depressed and this mental attitude may affect his desire for food and his powers of assimilation and hence his vitality and resistance to the infection may further suffer. Therefore attention to the mental condition is necessary and an attempt should be made to encourage the patient and to keep him from brooding over his unfortunate state. For this reason it is important that suitable and if possible entertaining work be provided for him and in all leper institutions it is advisable to keep every leper employed according to his capacity for work even though some can do very little. Healthy outdoor employment if not too strenuous may be beneficial toward recovery from the disease. Various industries agriculture and dairy farming related to the needs of the leper institution may be indulged in by many of the patients with less advanced lesions. The establishment of a school or a band of music theatrical performances etc are also of importance. An attempt should be made to have the leper lead as nearly as possible a natural life and to encourage him to forget his unfortunate condition and to feel that he is a useful member of the leper community in which he dwells. How much can be accomplished in this respect may be seen from a visit to the Government leper colony in the Philippine Islands which occupies the beautiful island of Culion. Here there have been collected since 1906 more than 12 000 lepers.

Bodaan has also described the conditions of a leper village settlement in Java where voluntary isolation is carried out which presents a good example of what can be done for lepers by tactful and sympathetic treatment.

As in all chronic wasting diseases the diet constitutes a most important feature in the treatment of leprosy. The waste of the tissues must not only be built up but the strength of the patient and his natural resistance to infection must be conserved as far as possible. Hence it is important for the diet to be of a proper nature and properly prepared as well as nutritious and sufficient in amount in proteins fats carbohydrates and vitamins. Fresh meat vegetables fruit and dairy products have a very important place in the diet of lepers. Although it has been suggested that fish should be avoided there appears to be no definite evidence that fresh fish has any unfavorable effect on the disease. Dutton points out that when the food supply consists mainly of fish or of salted fish a deficiency of some elements of diet may occur and that no fish except shellfish contains carbohydrates. Underhill Honeij and Bogert have pointed out that when leprosy patients are given calcium they tend to retain it to a very marked degree and they suggest that plenty of calcium should be supplied in the food as a therapeutic measure.

fact that the contagiousness of the affection is usually slight and that there are few instances that prove undoubted transmission of the disease from one person to another. However knowing that immense numbers of the bacilli are given off from ulcerations and other active lesions in the nose one should guard especially against the dissemination of leprosy bacilli from such sources.

Like tuberculous patients lepers may contaminate their immediate surroundings with their secretions and excretions. Hence these should be sterilized as in tuberculosis. Coughing sneezing and expectorating may cause dissemination of the disease. It should be recalled, also that the urine and perhaps the faeces may contain the causative organism. Especially then patients with bone lesions i.e., excoriations ulcers secreting wounds etc. both of the skin and mucous membranes should be strictly segregated. Patients with the maculo anaesthetic variety provided they have no demonstrable excoriated lesions in the nasal buccal or pharyngeal mucosa need not generally be strictly segregated but they should be required to take all precautions against the contamination of others. Continued association with leprosy individuals should be avoided. Rooms bedding wearing apparel cooking and eating utensils should be used only by the patient. They should be thoroughly sterilized by heat before being used by others. Even more careful precautions should be carried out than in pulmonary tuberculosis. Lepers should not be allowed to beg in the streets (as is common in some countries) or keep shops or handle food or other articles intended for sale. They should not also be allowed to frequent fairs and public places or hire themselves out as servants. Children born of lepers must be at once removed from the diseased parent and carefully observed subsequently for evidences of the disease.

### TREATMENT

**General Treatment**—As soon as the diagnosis of leprosy has been carefully made it is important that the patient should be placed in hygienic surroundings and that these be made as attractive for him as possible in connection with his isolation. In order that the feeling of isolation may be alleviated as much as possible it is usually better to allow him to associate with other individuals suffering with leprosy. Obviously this can best be accomplished in properly arranged leper colonies or institutions devoted to the care of lepers. He should be placed upon a sufficiently abundant and nourishing diet. Thorough cleanliness and hygiene of the skin should be maintained and clean underclothing frequently supplied. Pediculosis scabies ring worm infection with *Demodex folliculorum* and other cutaneous disturbances should be eliminated by proper treatment. Frequent bathing with plentiful use of soap is advisable and sodium bicarbonate may often be added to the warm bath for its cleansing properties. Certain natural baths in Japan were formerly thought to possess curative properties and in Hawaii the aromatic leaves of the eucalyptus tree were formerly placed

recreation play as great a role in the treatment of leprosy as in the treatment of tuberculosis

**Drug Treatment.**—Chaulmoogra oil or its derivatives has for many years been the standard treatment for leprosy. The oil (*Oleum gynocardium*) is obtained from the seeds of *Taraktogenus kur* in Burma and Assam and contains two unsaturated fatty acids (*Acidum chaulmoogricum* and *acidum hydnocardicum*). Formerly the oil was also prepared from the seeds of *Gynocardia odorata* but this oil is practically without action since the two fatty acids just mentioned are not contained in it. The oil is also obtained from the seeds of *Hydnocarpus wightiana* in southern India and *H. anthelmintica* in Siam and China. The pure oil may be administered by the mouth in doses of 5 to 60 minims 3 times daily. It may be taken with a lump of sugar or mixed with milk or vichy water. Some patients bear it comparatively well. In many others it causes gastritis and they are unable to assimilate large doses. This not infrequently necessitates cessation of the remedy. However many patients have improved strikingly after long continued ingestion of the oil. Engel Bey, who has had a large experience with leprosy in Egypt recommends oral administration in dosage of 30 drops of the purified oil solution under the trade name antileprol for a period of 3 to 4 years. Doses of the oil have also been successfully given in pill form. DeLangen (1936) recommends the following prescription for such pills

R̄	Oil Chaulmoograe	
	Cerae flavae	
	Sacch alb	aa 195
	Pulv rad glycyrrh	
	Ext glycyrrh.	aa 200
	M f pul No M	

He believes the best way to administer the pills is in ascending doses beginning with 5 pills 3 times a day and increasing 2 pills a day every third day. If symptoms relating to the stomach develop the course must be stopped for a week or two and then recommenced. The dose may be pushed as high as possible. The rather large pills of chaulmoogra oil that have been in general use contain 100 milligrams (Gr 1 5) of the oil. Other clinicians have employed the oil in gelatin capsules given directly after meals. While some still emphasize the value of the oral administration on account of the fact that so many patients cannot tolerate it and in some it causes toxic symptoms the oral administration has been largely abandoned. The irritant properties of the oils have been shown to be due especially to the decomposition products of their therapeutic constituents that is chaulmoogric hydnocarpic and goric acids. This decomposition takes place rapidly in the seeds and hence it is necessary to use only fresh oils from fresh seeds. The oil itself is quite stable and keeps fairly well under proper conditions of storage.



Every effort to improve the general condition of the patient should be made and particularly on this account a careful examination of the stools for intestinal parasites should be carried out and any parasites found present should be eliminated as far as possible by proper treatment. Ancylostomiasis and other intestinal parasitic infections are very common among lepers. Malaria and syphilitic infection should also be sought for and if either is present, treatment with quinin or arsphenamin, as the case may be, should be administered. In this connection it should be borne in mind that many lepers will give a positive Wassermann reaction even in the absence of coexisting syphilis. Either syphilis or tuberculosis may be associated with leprosy in the same patient. Constipation or diarrhoea or dysentery during the disease may also require special and proper treatment.

When attention has been given to these details of treatment as outlined above and the patient has been placed in favorable surroundings and given proper diet and kindly care many cases begin to improve without specific treatment. There is often an improvement in the general nutrition, a gain in weight, and sometimes even an improvement or disappearance of the lesions of the skin. Also the mental condition of the patient frequently becomes better, this feature being no doubt sometimes influenced by the fact that he no longer fears the detection of his ailment. However, usually this improvement is only temporary and fresh exacerbations of the disease occur.

A number of references are found in the literature to the spontaneous recovery of cases of leprosy. If specific treatment is given which subject will be discussed presently, the visible lesions may also disappear entirely and after a considerable period the leprosy bacilli may no longer be found in the excretions. McCoy, who has had a wide experience with the disease, states that when asked about the curability of leprosy he usually answers that he has seen a number of cases of recovery, but doubts if he has ever seen one cured. Throughout the course of treatment and observation of the patient it is important that he should continue to observe the general rules of health. Relapses after long periods of quiescence are frequent. If diet, work in the open air, rest, and sanitary surroundings are neglected and the resistance of the patient lowered thereby, the lesions and symptoms of the disease often reappear. We do not know whether climate plays any part in relation to treatment and we can only say that in some localities the disease shows no tendency to spread, while in others it does. Whether these differences are dependent upon temperature and moisture seem doubtful.

Hasseltine (1938) in emphasizing the need for institutional care and treatment in leprosy regards it as in every way as desirable as for tuberculosis. He reports that leaving out those that enter the national leprosarium at Carville in a moribund state, he can assure practically every patient admitted that improvement will take place in the first 3 to 6 months. Rest, good diet, and regular habits as to sleep, exercise and

intravenous use As very small intravenous doses of alepol may occasionally produce long and severe reactions it is desirable to begin the intramuscular and subcutaneous doses with 1 cc of a 3 per cent solution increased gradually twice weekly up to 5 cc or more or until saturation has been obtained It is better to use intramuscular injections until reactions cease before commencing intravenous injections

McDonald recommended the use of *ethyl esters* with the addition of 2 per cent iodine by weight and reported good results in Hawaii Rodriguez and Lara in the Philippines and Muir in India have also used extensively the iodized ethyl esters The dosage recommended in the Philippines is from 2 to 5 cc once a week intramuscularly The injection of the preparations *intradermally* has been used extensively first in the Philippines and later in India by Muir and is known as the infiltration method The esters are injected into the actual lesions of the skin by means of multiple small punctures raising a wheal not more than 1 cm in diameter One half to one drop is injected into each puncture and up to 5 cc may be injected into an area some 10-20 sq inches at a sitting The lesions so treated often become negative bacteriologically in 1 or 2 months The addition of 4 per cent creosote to the esters renders them less irritating

Hurwitz and Anderson (1936) have studied the value of *chaulphosphate* This preparation is said to be relatively nontoxic in that it is one fifth to one tenth as lethal for animals as alepol It is nonsclerosing in type and a water soluble chaulmoograte (na dichaulmoogryl  $\beta$  glycerophosphate) It does not cause immediate haemolysis in the blood cells and has been recommended for intravenous use in the treatment of the disease Anderson administered this drug to 20 patients in 0.5 to 1.0 gm amounts dissolved in sterile physiologic saline solution Treatments were given twice or thrice weekly While some lots of the drug gave no reactions others called forth severe febrile responses up to 40 C with nausea and vomiting These untoward effects however were transient and were believed to be due to the presence of carbonates and phosphates in the crude drug preparation used Cases treated showed marked improvement

A new chaulmoogra cholesterol complex has been prepared by Beranger and its value reported upon by Flandin (1938) He stated that given intravenously the drug is unusually effective and is especially valuable in lepra fever It has not been tried out however by other leprologists

Johansen has recommended as being less irritating the use of *benzocaine chaulmoogra* oil for intramuscular injection Three grams of benzocaine are added to 10 cc of olive oil and mixed with a stirring rod this is then added to 90 cc of chaulmoogra oil previously warmed on a water bath to 70 C the oil mass is then agitated in a flask until all remaining crystals of benzocaine are dissolved The mixture is filtered through filter paper and then heated on a water bath at 100 C for one hour Benzocaine goes into solution without increasing the volume of the finished mixture After experimentation to determine dosage and the most appropriate

More commonly the intramuscular, subcutaneous and intradermal injection of the oil has been employed. In the Philippine Islands in earlier years at the San Lazaro leper asylum subcutaneous and intramuscular injections of Mercado's formula formerly were employed by Heiser consisting of a mixture of 60 cc of chaulmoogra oil and camphorated oil and 4 gm resorcin. Injections were made weekly, commencing with 1 cc the dose being increased steadily according to tolerance. These injections however even when given into the gluteal muscles often gave rise to considerable pain and many patients show intolerance after a dose of only a few cubic centimeters has been reached.

Rogers recommended especially the oil from *Hydnocarpus wightiana* because its seeds were found to contain 10 per cent hydnocarpic acid which was nearly twice as much as obtained from other seeds and apparently this acid is the most powerful antileprotic one. A 3 per cent solution of sodium hydnocarpate from the oil of this plant has been placed upon the market. Its use intravenously was recommended. However thrombosis of the vein and phlebitis are apt to occur as a result of continued intravenous injections. More recently Rogers employed a 3 per cent solution given intramuscularly or subcutaneously twice weekly in doses commencing with 0.5 cc and increased by the same amount at each dose up to 5.0 cc or more.

Other authorities have preferred sodium morrhuate 1 cc combined with the gynocardate.

**Ethyl Esters**—Holman and Dean in their investigations in the Hawaiian Islands, for a number of years prepared and administered the ethyl esters of the fatty acids of the oil. The preparation is injected into the gluteal muscles once or twice a week in doses ranging from 1 cc to 5 or 6 cc (10 drops to 1 dram). Usually 1 cc is first given and the dose gradually increased. Treatment must be continued over prolonged periods 2, 3 or even 5 years with intervening periods of rest. It is now recommended that fortnightly determinations of the sedimentation rate of the blood should accompany the chaulmoogra therapy and when the rate is high treatment should be temporarily omitted.

A number of preparations of the ethyl esters of chaulmoogra oil have been placed on the market.

**Antileprotol** is an ethyl ester put up in capsules of 15 gr each and given intramuscularly or by the mouth twice weekly and in gradually increasing doses.

**Moogrol** consists of the ethyl esters of the entire fatty acids of the whole oil. The initial dose is 1 cc given intramuscularly and this is increased by the same amount in every second or third injection until 5 or 6 cc are reached depending upon the age and weight of the patient. Smaller amounts may be injected intradermally.

**Alepol** is prepared from the sodium salts of a selected fraction of the less irritating lower melting point, fatty acid of hydnocarpus oil. A 3 per cent solution can usually be given subcutaneously or intramuscularly without causing pain. A 1 per cent solution has been recommended for

**Other Drugs Aniline Dyes**—During the past 7 years attention has been attracted to the employment of aniline dyes in the treatment of leprosy. The selective affinity of such dyes for leprotic lesions combined in many cases with their powerful bactericidal activity of acid fast organisms *in vitro* raised considerable hopes for this form of treatment.

Trypan blue and fluorescin and methylene green were especially recommended. Ryrie in Malaya gave trypan blue injected intravenously in 25 cc doses of a 4 per cent solution, fluorescin in 10 cc of a 2 per cent solution and eosin in 25 cc of a 2 per cent solution. A definite diminution of the external manifestations of leprosy was observed. Scala of Messina administered intravenous injections of methylene blue to 11 lepers with complete failure of any favorable results.

Soluble fluorescin allied to mercurochrome has been employed in doses of 10 cc of a 2 per cent solution given twice weekly. However Emerson and Anderson (1934) have emphasized the toxicity of all these drugs and the dangers of repeatedly using high doses of them in human leprosy. They point out also the superiority of oral administration over their intravenous use. Denny Hopkins and Woolley some time ago reported that mercurochrome given intravenously once weekly to 44 lepers caused improvement in 16. However Hurwitz and Anderson (1936) who employed merthiolate another mercury fluorescent type of drug in 10 patients for over 2 months intravenously found no improvement in the leprosy and the drug was discontinued on account of the unfavorable symptoms such as pain in the back, stomatitis and albuminuria. Soule (1939) administered methylene blue intravenously to 11 lepers without any beneficial results.

Lowe (1939) has studied the effect of rubrophen which has been claimed to be of some value in the treatment of leprosy. However he was unable to confirm this. None of the cases chosen showed any improvement after the administration of the drug.

Anderson and his associates (1936) found that nephritis a common complication of leprosy is aggravated by dyes especially methylene blue and also by chaulmoogra oil preparations, metals and certain other drugs. In his opinion patients with damaged kidneys should not be given intensive specific treatment with renotropic agents.

The International Leprosy Congress resolved that the hopes regarding the value of these dyes has not been fulfilled. The dye treatment in leprosy cannot be considered to have reached the stage where favorable recommendations regarding it can be made.

**Protein Shock Therapy**—Many attempts have been made to treat leprosy by protein shock therapy. The favorable results produced by febrile reactions in leprosy have especially encouraged this method of treatment and intravenous and subcutaneous injections of various organisms have been made.

Many so called specific products whether of the nature of extractives as leprolin or nastin or of bacterial vaccines have been tried with results which have not tended to gain the confidence of conservative clinicians. The product which has been given most general trial is nastin. This is a neutral fat extracted from a streptothrix growth obtained by Deycke from leprous nodules. It is combined with benzoyl chloride and is contained in ampoules containing from one half to one fifth of a milligram.

Wise and Minnett treated 244 cases with nastin for periods of from 1 to 2 years the treatment having been at first supervised by Deycke himself. It was stated that nodular cases did not seem to be improved and that anaesthetic leprosy was not apparently influenced. Recent reports have not confirmed its value.

regions for repeated injections, it was ascertained that the maximum average, comfortably tolerated dose was the semiweekly injection of 5 cc into the deltoid regions, alternating with 8 cc into the buttocks and this was adopted as routine. Certain muscular lepers tolerate 15 cc twice weekly with no reported discomfort other than that to be expected from the size and pressure of the mass of oil. It was found that the oil completely absorbed within 48 hours in the majority of patients and rarely any evidence of the injection was noted after the third day. The mixture is best given at body temperature as this allows the oil to pass freely through a medium sized needle, thus giving only a minimum of pain from the puncture.

Benzocaine which is ethylis aminobenzoate of the U S P is a local anaesthetic soluble in oil and is not a habit forming drug. Its use in the above described way makes it possible for the unfortunate leper to absorb the maximum amount of chaulmoogra oil without pain and without disgust.

This preparation has been employed at the U S national leprosarium at Carville La since 1928. Hasseltine (1938) has reported that nearly all of the 365 patients were treated mostly by the benzocaine chaulmoogra oil intramuscularly or the hydnocarpate esters.

At the International Leprosy Congress (1938) it was resolved that chaulmoogra oil from *Hydnocarpus* species and its ethyl esters administered intramuscularly subcutaneously and intradermally remains the most efficacious for the special treatment of leprosy that no proprietary preparation of *Hydnocarpus* oil or esters or any other proprietary preparation at present on the market is more effective than the pure oil and esters prepared in institutions. It was however emphasized that treatment of *Hydnocarpus* oil or esters should be discontinued at the onset and during the course of the lepra reaction (lepra fever).

Wade points out that patients undergoing routine medication with the oil or esters register a variety of complaints particularly when the larger doses are reached. The conditions complained of are particularly

(1) The immediate effects of the drug—choking and dizziness which appear immediately after injection. (2) local effects in the lesions—induration and abscess formation. (3) general effects—fever and headache. (4) effects upon the respiratory system—cough, chest pain, chest oppression and haemoptysis which may not necessarily depend upon pulmonary disease. The most common complaints made by the patients at Culion are cough, chest oppression, fever, malaise and weakness in the order mentioned. Pulmonary tuberculosis is one of the chief contra indications to the treatment. Doses of the oil large enough to affect the leprotic lesions in tuberculous cases are decidedly harmful. Active treatment is contra indicated when acute or advanced chronic nephritis is present. If the kidney involvement is not marked the purified oil may be tried. The treatment should also not be administered to anaemic and debilitated individuals. Those with marked cutaneous lesions do not as a rule tolerate medication well. Proper attention to the food and exercise of the patient has been found to be quite as important as the drug itself. Choking appearing immediately after an injection may be so severe as to be alarming. To relieve an attack the patient is given a drink of water and then made to lie down quietly. In a very few cases the paroxysm of coughing may be so severe as to require a hypodermic injection of morphine and atropin.

Addendum—G W McCoy (1942) writes that there has been wide spread belief among the members of the medical profession that chaulmoogra oil and its derivatives are valuable—specifically curative agents—in the treatment of leprosy. He adds this is in marked contrast with the views expressed by many experienced students of the disease when the subject is discussed privately. His own observations have led him to the conclusion that the oil and its derivatives are of little or no curative value and that the unpleasant side effects probably outweigh any advantage to the patient that might possibly accrue from their use. He quotes from a number of authorities who apparently concur in this opinion.

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However Muir has employed a suspension of Kederosky's acid fast bacillus intravenously and obtained favorable, though temporary results due to the protein shock induced \*

Manson Bahr (1940) injected typhoid and para typhoid vaccine intravenously and thought the improvement which resulted was due to the protein shock. It seems not unlikely that the temporary beneficial effects so often observed from various other vaccines, sera and bacterial emulsions are due to the shock like reaction produced by the foreign protein contained in them. Diphtheria antitoxin has been employed for this purpose in Bangkok by Collier (1940). Cobra venom has also been recommended by Chopra & Chowhan (1939) on nerve leprosy.

*Ephedrine* in doses of  $\frac{3}{4}$  to 2 gr by the mouth in hard gelatin capsules has been especially recommended by Muir to relieve the nerve pains of leprosy.

*Adrenalin* has also been employed 3 minims being injected in saline solution intravenously along the course of the affected nerve. *Ephedrine* may also be given in the same manner suspended in 10 cc or  $\frac{1}{2}$  per cent carbonate solution.

**Thiamin Chloride (Vitamin B<sub>1</sub>)**—Badger and Patrick (1938) during 6 months treated 10 patients who had rather severe acute leprous neuritis of the peripheral nerves with intramuscular injections of thiamin chloride (vitamin B<sub>1</sub>). The injections were begun as soon as possible after the onset of symptoms or when the patient first complained of pain. The procedure followed was to give 300 international units once a day by intramuscular injection and twice a day by mouth. In the 7 cases in which the injections were begun on the day of onset the pain disappeared completely 24 hours after the first injection in four 48 hours in one and on the fourth day in another. Definite diminution in the swelling occurred about the time the tenderness disappeared.

*Antimony* has been employed extensively in leprosy. It appears to be of particular value in controlling the condition known as the lepra reaction. Muir has particularly advised intravenous injection of *tarlar emetic* for reducing the lepra reaction caused by iodine. More recently the colloidal form of *oscol stibium* 1 cc to be given at intervals of 3 days has been recommended.

Cochrane (1938) has investigated the question of why various workers report failure to control the lepra reaction by treatment with the antimony products. He believes that the confusion in the literature is due to the inadequate appreciation of what is really meant by the lepra reaction and what for want of a better name has been described as the reaction state (an allergic condition seen in tuberculoid leprosy) is confused with the true lepra reaction. Further the lepra reaction can be subdivided into acute sub acute and chronic stages. Antimony products are only of definite value in the acute lepra reaction, to bring the temperature down. He found it did this in all cases after 4 to 6 injections. He also found by experimentation that a pentavalent antimony product

Grasset and Davison (194) have employed injections of a killed non acid fast strain of tubercle bacillus and believe it of value in the treatment of patients with the neural type.

fouadin was as effective as potassium antimony tartrate and easier of administration

**Potassium Iodid**—Muir when in India reported good results with large doses of potassium iodid given twice weekly and gradually increasing the dose until the maximum is reached so that 120 or even 240 grains were given in one dose

It is well known that the iodids and especially potassium iodid often produce a marked reaction in leprosy. A number of observers have considered this salt useful especially in connection with the diagnosis of the infection because it was found that the nasal catarrh produced by its use often facilitated the search for lepra bacilli in the nasal secretions. Some workers have regarded the apparent exacerbation of the disease produced by the drug as dangerous. Muir however considers that the reaction produced by potassium iodid is not necessarily harmful and that the breaking down of leprous tissue caused by the administration of the drug if the dosage is wisely regulated may be one of the most beneficial processes possible in the treatment of the disease. Potassium iodid does not however lend itself to use in mass treatment. It is advisable to begin with small doses and to gradually increase these according to the tolerance of the patient. Less than 1 grain may cause a febrile reaction which will last two or three weeks while later on as the condition improves such massive doses as 240 grains a day may be taken without effect. Muir thought that potassium iodid is a most useful therapeutic agent in all stages of leprosy. In cases in which a considerable amount of leprous granulomatous tissue has been formed the breaking down of this tissue by potassium iodid apparently induces a considerable degree of immunity and these two factors the breaking down of leprous tissue and immunity combined are in his experience more powerful therapeutic agents in leprosy than any others which he has seen. The reactions after injection of the iodid are the following: (1) swelling up and erythema of the existing lesions (2) the appearance of flesh rose colored nodules which are often painful (3) fever not always present (4) marked acceleration of blood sedimentation and (5) apparent granulation of lepra bacilli in the lesions. If rose-colored nodules appear and disappear again in a few days the physician can press the treatment with some assurance as their disappearance is a sign of immunity and when this is present the breaking up of granulomatous tissue and getting free of bacilli in the general circulation will not cause further dissemination of active disease but a gradual healing up of the lesions. While the treatment may be suitable for all the stages and types of leprosy in many cases the larger doses produce swelling of affected nerves and induce pain and tenderness in them. In the second and third stages of the disease in which lepromatous tissue is abundant even the smallest doses generally produce all the reactions referred to above.

However the International Congress of Leprosy (1938) emphasized that the use of this drug frequently causes disastrous results and its use is therefore to be discouraged for the purposes of diagnosis and treatment or as a test of recovery unless in very skilled and experienced hands.

**Arsenobenzol**—This drug has been employed in cases in which there has been coexisting syphilis. Radna (1939) emphasizes the elimination of leprosy bacilli in the urine after the administration of arsenic in 9 cases in which the urine was negative before treatment with novarsenobenzol. In these cases it was positive for leprosy bacilli after the treatment.\*

**Thermal Treatment**—Nocht and Velasco have submitted leprosy patients to long repeated artificially produced rises of temperature for a duration of from 8 to 10 hours and as high as 40 C (104 F) and more. The treatment was well tolerated by the leprous patients and apparently

Faget and Poggé (1943) regard promin as the most valuable of all the sulfonamides in the treatment of leprosy although apparently none has been marketed yet by the time it is written by the tenacious injectors.



did not produce any dangerous or inconvenient after effects in the lepers who were in good health. Most of the cases, however, appeared not influenced even in those in which the treatment was repeated and prolonged. Only in special cases was there marked improvement. Ross (1938) at the Carville leprosarium treated 5 patients with 3 courses of weekly treatments during each of which the patient's rectal temperature was raised to between 105° and 106° F for from 1 to 3 hours, and in one patient for 5 hours. In some instances there was an impairment of the renal function with albuminuria and casts. The renal function tests however, showed no evidence of permanent damage to the kidneys. No favorable results were reported from the treatment.

Johansen (1929) has also reported on 18 leprosy patients treated with a total of 164 fever treatments induced by means of the Kettinger hypertherm with maintenance of the patient's temperature as a rule to 105°F—106°F for 5 hours. In 15 of the 164 treatments complications necessitated the termination of the treatment before the full time. Among the complications were shock, nephritis and delirium. In 66 per cent hyalin and granular casts and in most of them also albumin were present. Temporary loss on the average of 4.4 lbs. of weight were reported. The results were very unsatisfactory for of the 15 patients, 13 were worse after the treatment and 2 remained stationary.

**Intranasal Therapy**—Pinkerton who has had many years experience in the study and treatment of the pathological conditions of the nose, throat and larynx in leprosy, believes that none of the many remedies described, including chaulmoogra oil, act as a specific in local or topical treatment. There should be hygienic care of the mucous membranes. Such treatment resolves itself largely into methods of cleansing and the accomplishment of drainage of the nose. These appear to assist uncomplicated healing and certainly give comfort to the patient. He has used a spray of chaulmoogra oil directly into the larynx without favorable results. The use of bland oils by inhalation of the fine spray seems agreeable to the patient. The treatment of leprosy of the upper part of the respiratory tract should be much the same as that of treating the same parts in the tuberculous patient and since the condition of the nose, throat and larynx reflects to a great extent the general condition of the patient the treatment should in the main be directed toward improving the general condition. The patient who rests his larynx has less cough and irritation than one who indulges in talk to an amount which abuses his larynx.

Others however have recommended ionization for the treatment of actual nasal lesions: the 1 per cent sodium salts of *H. nighiana* with alepol or potassium iodid. A current of 20 to 30 ma. for 20 to 30 minutes is applied to each nostril separately and 3 or more sessions at bi-weekly intervals are recommended to clear up the local infection and to reduce the number of leprosy bacilli in the discharge.

Other symptoms have to be treated as they arise. Laryngeal affections may require insufflation of cocaine. Leprotic iritis may be extremely difficult to treat and often atropine drops are of little avail. In these

cases hyoscine (scopolamine) hydrobromide  $\frac{1}{2}$  per cent solution may be used in the form of drops it usually gives relief For the offensive nasal discharge the following nasal lotion has been found useful

R Sod chlorid	gr xxii ( 1 42 gm )
Sod bicarb	gr xxii ( 1 42 gm )
Pot chlorid	℥ ii ( 7 78 gm )
Calc phosph	℥ ss (15 55 gm )

Half oz to be used with  $\frac{1}{2}$  pint of warm water as a nasal douche

For the eye lesions Muir has recommended injections of trypan blue (Grubler) A 1 per cent solution in normal saline is injected subconjunctivally and at the same time 0 cc of a 1 per cent solution injected intravenously twice weekly according to the tolerance of the patient He thinks the dye seems to exert a sedative action upon leprous granulomata see p 865

Vaile (1939) and others state that chaulmoogra oil and its derivatives are liable to provoke dangerous reactions in lesions of the eye and advise that they should not be used when ocular complications are present

Radium may be employed for lesions about the eye as well as in the mouth When leprous nodules appear on the cornea the extent of the leproma may be arrested sometimes by division of the cornea on the pupillary side of the lesions Brockmann reported that the bacilli do not traverse the cicatrix Tarsorrhaphy for ectropion of the lower lid iridectomy for iritis or synechiae may be necessary Sometimes it is also necessary to perform tracheotomy for laryngeal stenosis by which wonderful relief of intense pain and distress is often obtained

Surgical treatment is frequently of value in nerve stretching for the relief of intractable leprous neuralgia but the results are sometimes disappointing Lowe (1939) reports that surgical removal of the sheath of the ulnar nerve has been performed in a number of leprosy patients suffering from acute neuritis The procedure has been found to be of great help in relieving the pain and it appears to be effective in the prevention or minimisation of deformity

For the treatment of perforated ulcers amputation of the area involved is recommended The existence of leprosy does not materially interfere with the success of surgical operations with early healing Cutaneous lesions nodular infiltrations and ulcers often respond favorably to exposure to the roentgen ray Wise (1938) recommends that infiltrated lesions and deep seated ulcers should be treated with filtered rays the average dose being 1 Holzknecht unit (350 r) skin distance filtered through 3 mm of aluminum screen once every 3 or 4 weeks Frequent heat baths with sodium bicarbonate dissolved in water are beneficial in some cases Necrosis can be treated by ultra violet rays This has been reported upon favorably at Carville and in the Dutch East Indies

When one or several lepromata or leprous macules are present and there have been no constitutional signs of a general invasion it is recommended to excise them completely Wayson (1939) has reported the

removal a few weeks after their appearance of 3 leprous nodules from the head of a French priest who worked in a leper settlement in Hawaii. This apparently prevented any further spread of the disease during four years. Wayson thought the infection in the patient was presumably transmitted to the skin of the head directly from his fingers after handling the patients. He mentions a similar case in a child who was operated upon by McCoy and in whom no further manifestations of the disease occurred in 16 years. Lowe (1939) also reports total excision of the skin lesions in a number of early cases of the neuro macular type of leprosy. The results are such that if cases are suitably selected complete excision of the lesions is not likely to be followed by a relapse at least in a certain percentage of the cases. The period of observation however in the majority is too short to allow any definite conclusions to be drawn.

In view of occasional reports of the successful removal of solitary lesions of nerve leprosy in particular the International Journal of Leprosy (1939) invited correspondence on the subject as a result of which 19 cases were tabulated, with the following results—No relapse in 12 anaesthesia of the scar only in 2 new lesions appeared elsewhere in 2 and 3 were not reexamined. It is pointed out that such slight lesions often yield readily to medical treatment.

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